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DOTTORATO DI RICERCA IN
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**“Congenital and aquired myopathies in dogs:
case series”**

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INTRODUCTION

Diseases affecting muscles and the neuromuscular junction are commonly overlooked when evaluating a dog with lameness. Orthopedic disorders occur more frequently than neuromuscular disorders; however, when a diagnosis cannot be reached following a careful orthopedic evaluation, neuromuscular pathologic conditions should be considered (Shelton 2006). Poor attempts, especially in small animals, have been made to evaluate the prevalence of neuromuscular disorders in veterinary medicine, whereas various studies explore their epidemiology in specific geographic areas (Emery et al. 1991; Merlini et al. 1992; Hughes et al. 1996; Darin & Tulinius 2000; Aleman 2008; Graziano et al. 2015).

The clinical signs of neuromuscular disease can be enigmatic. The cardinal sign of neuromuscular disease is weakness, but some neuromuscular diseases do not exhibit weakness, whereas a number of non-neuromuscular diseases will present with weakness as major clinical feature (Amann 1987). Affected individuals with muscle or neuromuscular junction disease may present with lameness, limb contractures or more generalized musculoskeletal abnormalities, such as a crouched stance and stiff, short-stridden gait. Muscle tone and peripheral reflexes are normal or reduced and there may be significant muscle atrophy in chronic myopathies. In some individuals, weakness of the pharyngeal, laryngeal and esophageal muscles may result in dysphagia, dysphonia or regurgitation. Pyrexia may be present in inflammatory myopathies or be associated with aspiration pneumonia as a result of mega-esophagus (Shelton 2006).

In human medicine, the approach to the diagnosis of myopathies follows a well-determined algorithm. In presence of proximal muscle weakness and atrophy,

early fatigue and/or presence of flexural deformities simple musculoskeletal pathologies are ruled out with clinical and radiographical evaluation. Then firstly, serum muscle markers are determined: if they result raised compared to the physiological threshold a muscular disorder should be considered. Electromyography (EMG) and nerve conduction studies (NCS) are the most useful to exclude denervation disorders. In congenital myopathies, the EMG is typically normal or shows myopathic features, but occasionally EMG changes that appear neurogenic can be seen with severe neonatal weakness or in distal muscles later in the disease course. Nerve conduction studies are normal (North et al. 2014). Specific investigations such as repetitive nerve stimulation or single fibre EMG are important to exclude myasthenic syndromes, although some congenital myopathies can be associated with neuromuscular junction abnormalities. Investigations other than muscle biopsy are rarely specific for myopathies, but are widely used to exclude other possible diagnoses. Imaging techniques, such as radiographs, computed tomography or resonance magnetic imaging, and ultrasound have assumed increasing importance in the diagnostic approach for patients with muscle disease and show specificity for several genetic entities. The degree of involvement of the muscle can be suspected on clinical grounds, it may be very helpful to utilize muscle imaging (MRI, ultrasound, or CT) to estimate the degree of involvement. MRI should be regarded as a gold standard technique (Bönnemann et al 2014).

Indeed, over recent years muscle ultrasound and magnetic resonance imaging (MRI) have been increasingly used to differentiate between different forms of congenital myopathy. Selective muscle involvement on MRI can be suggestive of

a specific disease gene, however the specificity is variable and imaging is usually interpreted in conjunction with clinical phenotype and results of muscle biopsy to prioritize gene testing. In the future, once data has been collected to determine the specificity of patterns of muscle involvement muscle MRI may be used in conjunction with clinical features to guide genetic testing prior to muscle biopsy when a congenital myopathy is suspected. Muscle ultrasound is a practical way to image muscle that does not require general anaesthetic and can be performed at the bedside. However its utility is dependent on the expertise and experience of the ultrasonographer. Muscle ultrasound can also be helpful in recognising possibly neurogenic changes and in selecting an appropriate muscle for biopsy. (North et al. 2014)

In veterinary clinical practice, the simplest and most routine diagnostic test for muscle disease is blood chemistry. A complete blood cell count, serum chemistry panel (including creatine kinase levels and electrolytes), and urinalysis should be evaluated in every animal with a suspected neuromuscular disease. (Glass & Kent 2002)

Enzyme activities that may be increased due to muscle disease are aspartate transaminase (AST/GOT), creatine kinase (CK/CPK), and lactate dehydrogenase (LD/LDH). These intracellular enzymes are released when the muscle cell membrane is injured. They are specific for diseases that affect the muscle cell portion of the motor unit. The half-life of these enzymes in the blood is specific for each markers so that an increased activity can be a good indicator not only of a muscle damage but also of its duration. The half-life of CK is only a few hours, whereas that of AST is several days. A number of cautions are necessary to

prevent misinterpretation of increased activity of muscle enzymes. When available, muscle biopsy remains the most useful diagnostic tool in the diagnosis of muscle disease, both in human and in veterinary medicine. Nerve biopsy can also be performed on either mixed or sensory peripheral nerves with little effect on the performance of an animal. This can be useful in the diagnosis of peripheral nerve disease. As in human medicine, in animals EMG studies represent the state of the art in neuromuscular diagnostic techniques. EMG can be performed to determine the nerve conduction velocity (sensory or motor), the presence of abnormal electrical activity in muscle (due to denervation, inflammation, or hyperexcitability of the muscle cell membrane), and the ability of the synapse to function (evoked motor action potentials and the decremental response) (Amann 1987).

ANATOMY AND PHYSIOLOGY

A typical neuron has four morphologically defined regions: the dendrites, the cell body, the axon, and the presynaptic terminals of the axon. These four anatomical regions are important in the major electrical and chemical responsibilities of neurons: receiving signals from the presynaptic terminals of other neurons (on dendrites), integrating these often-opposing signals (on the initial segment of the axon), transmitting action potential impulses along the axon, and signaling an adjacent cell from the presynaptic terminal. The *cell body* (also called the *soma* or *perikaryon*) plays a critical role in manufacturing proteins essentials for neuronal function. The cell body usually gives rise to several branchlike extensions, called *dendrites*, whose surface area and extent greatly exceed those of the cell body. The dendrites serve as the major receptive apparatus of the neuron, receiving signals from neighboring neurons. The cell body also gives rise to the *axon*, a tubular process that is often long. The axon is the conducting unit of the neuron, rapidly transmitting an electrical impulse (the action potential) from its initial segment at the cell body to the other end of the axon at the presynaptic terminal. Large axons are surrounded by a fatty, insulating coating called *myelin*. In the peripheral nervous system, myelin is formed by *Schwann cells*. The myelin sheath is interrupted at regular intervals by spaces called *nodes of Ranvier*. Axons branch near their ends into several specialized endings called *presynaptic terminals*. When the action potential rapidly arrives, these presynaptic terminals transmit a chemical signal to an adjacent cell, usually another neuron or a muscle cell. The site of contact of the presynaptic terminal with the adjacent cell is called the *synaps*. It is formed by the presynaptic terminal of one cell (presynaptic cell), the

receptive surface of the adjacent cell (postsynaptic cell), and the space between these two cells (the *synaptic cleft*). The receptive surface of the postsynaptic cell contains specialized receptors for the chemical transmitter released from the presynaptic terminal. Neurons, as with other cells of the body, have an electrical potential, or voltage, that can be measured across their cell membrane (*resting membrane potential*). The resting membrane potential is the result of the differential separation of charged ions, especially sodium (Na^+) and potassium (K^+), across the membrane and the resting membrane's differential permeability to these ions as they attempt to move back down their concentration and electrical gradients.

The *neuromuscular synapse*, also known as *neuromuscular junction* is a chemical synapse between a motor neuron and a skeletal muscle cell (fiber). The presynaptic side of the synapse is made up of the terminal portion of the motor neuron. This presynaptic terminal has a swelled, button-like appearance and is also called a *synaptic bouton*. The synaptic *bouton* contains a large number of membranous storage vesicles, called *synaptic vesicles*, which contain the chemical neurotransmitter substance, in this case *acetylcholine*. Directly opposite the face of the presynaptic terminal, the postsynaptic muscle cell membrane contains receptor for the acetylcholine transmitter. In this focal region the membrane has a series of invaginations, called *junctional folds*, that increase the surface area where acetylcholine receptors can reside. Because the neurotransmitter is found only on the presynaptic neural side of the neuromuscular junction, transmission can go only from neuron to muscle, not in the reverse direction. The function of the neuromuscular junction is to transmit a

chemical message unidirectionally between a motor neuron and a skeletal muscle cell (fiber) with a frequency established by the central nervous system. The arrival of an action potential at the motor neuron terminal triggers the release of the acetylcholine transmitter, which then binds with acetylcholine receptors on the postsynaptic muscle fiber membrane. This leads to the genesis of an action potential along the muscle fiber membrane that ultimately leads to contraction of the fiber.

All body movement is the result of contraction of skeletal muscle. When activated by a motor nerve, a skeletal muscle can only shorten. Each muscle belly is made up of differing numbers of *muscle fibers*, the outer limiting membrane of the fiber is called *sarcolemma* that consists of a true cell membrane, called the *plasma membrane*, and of an outer polysaccharide layer that attaches to the tendons at the cells' extremities. Each muscle fiber is innervated by only one motor neuron, with the neuromuscular junction region located near the middle of the fiber, relative to the ends. Each fiber contains several hundred to several thousand *myofibrils* arranged in parallel along its length and each myofibril is made up of a linear series of repeating *sarcomeres*, the basic contractile units of the muscle fiber. The sarcomere has a disk at each end, called *Z disk*. The sarcomere contains four types of large protein molecules responsible for muscular contraction, three of which are polymerized. Numerous thin protein filaments, called *actin*, are attached to the Z disks and extend toward the center of the sarcomere. Each actin filament consists of two intertwined, helical strands of actin protein and two such strands of *tropomyosin* protein, all bound together as a larger helical complex. Also located intermittently along the tropomyosinactin strand are complex globular

protein molecules called *tropoin* that can bind tropomyosin and actin and that have an affinity for calcium (Ca^{2+}) ions. Suspended between and parallel to the actin thin filaments are thicker filaments of *myosin* protein polymers. A myosin molecule contains a tail of intertwined helices and two globular heads that can bind both adenosine triphosphate (ATP) and actin. Beneath the plasma membrane of the muscle cell lies the *sarcoplasmic reticulum*, an intracellular storage organelle that forms a reticulated network around the myofibrils. This extensive storage sac sequesters Ca^{2+} ions in relaxed muscle and is analogous to the smooth endoplasmic reticulum in other cells. Located perpendicular to the long axis of the muscle fiber, there are tubes of plasma membrane formed by periodic invaginations of the sarcolemma. These *transverse tubules*, or *T tubules*, traverse the diameter of the muscle fiber. The T tubules snake around the myofibrils, forming junctions with the network of sarcoplasmic reticulum that surrounds the myofibrils. These tubules are filled with extracellular fluid and are important because they allow the electrically excitable plasma membrane of the muscle fiber to carry the depolarization of the action potential to the interior of the fiber.

Skeletal muscle cells have resting membrane potential, as do neurons, and the muscle cell membrane can be depolarized by synaptic transmission at the neuromuscular junction. At this junction, the acetylcholine released by the motor neuron activates nicotinic acetylcholine receptors on the sarcolemma of the muscle cell. The resulting depolarization is sufficient to open enough voltage-gated Na^{+} ion channels, also found at the junctional sarcolemma, to trigger a muscle fiber action potential. Thus, it is at the sarcolemma of the neuromuscular junction that muscle fiber action potentials are generated. Once an action potential

is generated near the midpoint of the muscle fiber, it spreads in both directions along the length of the fiber. The action potentials are also transmitted to the interior of the muscle fiber along the T tubules. This allows the action potential to reach the location of the sarcoplasmic reticulum even in the innermost regions of the muscle fiber.

The sarcomere is changed from its relaxed state to the shorter, contracted state when Ca^{2+} ions become available to the sarcomere. In the presence of Ca^{2+} ions and a sufficient source of ATP, the actin thin filaments are pulled in parallel along the myosin thick filaments by the repetitive movement of the myosin molecule heads, thus shortening the sarcomere. Because each myofibril is made up of a linear series of repeating and connected sarcomeres, the net result is the physical shortening of the distance between the two ends of the muscle: the muscular contraction. (Cunningham et al. 2007)

DEFINITION

The term “neuromuscular disease” refers to a broad category of pathologic conditions affecting nerve and/or muscle. Amann (1987) define neuromuscular disease as any entity that interferes with the normal structure and function of the motor unit. Neuromuscular diseases can be conveniently classified and studied according to the part (or parts) of the motor unit on which the disease’s primary effect is exerted (Amann 1987).

The neuromuscular system is a conduit for transmission of information from the central nervous system to the musculoskeletal system. Consequently, an abnormality in any portion of the lower motor unit can result in clinical signs of neuromuscular disease (Glass et al. 2002). For instance, neuropathies affect some aspect of the motor neuron cell body, axon, or telodendron. Spinal muscular atrophies affect the cell bodies located in the gray matter of the spinal cord; these conditions may also be termed motor neuron or ventral horn cell diseases. Radiculopathies affect the axons or their myelin sheaths in the spinal roots, whereas peripheral neuropathies affect the axon, its telodendron, or the myelin sheath in the peripheral nerve and muscle. When a pathologic process initially affects the telodendron or distal axon, it may be referred to as a dying back disease. Diseases that affect the neuromuscular termination are referred to as junctionopathies. A myopathy is a neuromuscular disease that primarily affects the muscle itself. Those diseases that have primary effects on both nerve and muscle are referred to as neuromyopathies. Any disease of any part of the motor unit will be reflected in a partial or complete loss of function in the muscle itself.

Muscle that is removed from the trophic influence of its motor neuron undergoes atrophy (termed neurogenic atrophy) (Amann 1987).

Neuromuscular disease may be either congenital or acquired. Congenital neuromuscular disease is any disease that an animal is born with or acquires as a result of some factor relating to birth. It may be an inherited entity or the effect of some in utero influence. Acquired neuromuscular disease is more common than congenital disease and often affects older dogs (Amann 1987).

Inherited peripheral neuropathies in dogs and cats have rarely been documented, and the mode of inheritance often can only be speculated. Furthermore, the specific genetic defect has not been identified for any inherited peripheral neuropathy in pets. These neuropathies can affect autonomic nerves, sensory nerves, and motor fibers alone or in combination (Coates et al. 2004).

Inherited peripheral neuropathies are classified as motor and sensory (mixed) neuropathies, primary sensory neuropathies, and neuropathies related to inborn errors of metabolism, including storage disorders. Pathologic studies further subdivide mixed and primary sensory neuropathies into those that affect the central nervous system (CNS) and the peripheral nervous system (PNS) as in central-peripheral distal axonopathy and those limited to the PNS. The latter are further subdivided into myelinopathies or axonopathies (Coates et al. 2004).

Muscle disease may be subdivided into six broad categories on the basis of sets of fairly distinctive clinicopathologic characteristics: atrophies, dystrophies, inflammatory myopathies, metabolic myopathies, "congenital" myopathies, and disorders of neuromuscular transmission (De Girolami et al. 1982).

DIAGNOSTIC TOOLS

A diagnostic plan for an animal with suspected neuromuscular disease should include a complete neurologic examination and minimum database (complete blood count [CBC], serum biochemistry panel, urinalysis, thoracic radiographs, and abdominal ultrasound). Electrophysiologic evaluation and muscle/nerve biopsies are essential considerations in the accurate diagnosis of most diseases affecting the neuromuscular system.

Specific abnormalities include anemia, hypo- and hyperglycemia, hypo- and hyperkalemia, hypo- and hypernatremia, hypo- and hypercalcemia, hypophosphatemia, and hypomagnesemia. If not part of the biochemical panel, serum creatine kinase (CK) activity should be measured. In addition, baseline testing in any dog or cat with clinical neuromuscular disease should include measurement of serum cardiac troponin I and plasma lactate concentrations and determination of thyroid status (Shelton 2010).

CBC may reveal abnormalities, such as a stress leukogram supportive of hyperadrenocorticism or a mild normocytic, normochromic, non-regenerative anemia supportive of hypothyroidism. The chemistry panel may reveal electrolyte abnormalities, ionic imbalances, and blood glucose aberrations, which may be responsible for myopathic signs (Platt et al. 2004).

Muscle enzymes are often isoenzymes of enzymes that occur in other tissue as well. The enzymes aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) are also present in hepatocytes, and these two plus creatine kinase (CK) are present in cardiac muscle (Amann 1987; Blot 2005).

Evaluation of serum CK concentration should be a part of the neuromuscular minimum database and may be indicative of active muscle disease in canine and feline patients. In muscle, CK functions in making ATP available for muscle contraction by the phosphorylation of ADP from creatine phosphate. The serum half-life of CK is short, lasting only 6 hours; a persistent elevation of four to five times the normal level in two tests performed between 24 and 48 hours of each other is an indication of a recent and active muscle lesion. CK may be normal in the presence of muscle disease, thus muscle diseases should not be ruled out based on a normal CK concentration. Serum CK may also be mildly elevated in the absence of neuromuscular disease related to factors like exercise, recumbency, or trauma (e.g., needle injections) or markedly elevated in anorexic cats. Elevations of CK are most dramatic in the muscular dystrophies or with myonecrosis (100 times normal); moderately elevated in inflammatory myopathies (10 times normal); or normal or only mildly elevated in other diseases, such as myotonia congenita. Elevated levels of serum AST, and LDH can also be compatible with muscle disease; however, as is the case for CK, they are not disease specific and can be normal in some myopathies (Platt et al. 2004).

CK and AST have very different kinetics, which need to be taken into consideration when diagnosing the extent and time frame of muscle damage. AST takes up to 24 hours to peak and several days to return to baseline. CK tends to be used for acute monitoring of muscle enzymes and AST for evaluation of changes over a longer period (Moses et al. 2007).

CK activity in blood is ultimately the most useful muscle enzyme measurement and can be used to follow the course of a muscle disease as well as monitor response to treatment or exacerbation of the disease (Amann 1987)

However, tremendous variability occurs, based on age (the younger the animal, the higher the value), breed (the smaller the dog, the higher the value), motor activity (exercise produces higher value), recent intramuscular injections, and sampling methods. Although some myopathies are associated with dramatic persistent elevations, numerous myopathies cause little, transient, or no elevation at all. Therefore, as previously stated, a normal CK level should not exclude a muscle disease from the differential diagnosis (Blot 2005).

Intramuscular injections of drugs can cause an increase in CK activity with a peak at about 4 hours, consistent increase for about 24 hours, and return to baseline by the third day. Serum CK activity can be increased as much as 12-fold in animals in prolonged recumbency and can also be increased during and after surgery, peaking within 6 to 12 hours. Electromyographic procedures can result in increased activity. Anorexia in cats may be associated with moderate to marked increases in serum CK activity and should not be considered diagnostic of myopathy until feeding has resumed. If the serum CK activity is persistently increased and external causes can be eliminated, an underlying neuromuscular disease should be considered (Shelton 2010).

Specific blood tests include a corticotropin stimulation test in combination with an endogenous corticotropin assay for hyper- and hypoadrenocorticism; a combination of serum total thyroxine (T4), an endogenous thyroid-stimulation hormone (TSH) assay, and free T4 levels for hypothyroidism; and serum antibody

titers for infectious diseases, such as *Neospora caninum* and *Toxoplasma Gondii*. Arterial blood gas analysis performed before and after exercise normally reveals significant elevations of blood pH and PaO₂, along with significant decreases in PaCO₂ and bicarbonate concentrations. Although the anion gap can be expected to increase as well after a period of exercise, it does not reach clinically significant levels (Platt et al. 2004).

Resting and post-exercise plasma lactate and pyruvate concentrations should be measured as a screening test in all animals with suspected myopathic causes of weakness and collapse, especially in those with signs related to activity. Although not as ideal, pre- and postprandial lactate and pyruvate analysis can be performed when rest and subsequent exercise of the patient are not possible. Blood for lactate concentration should be collected into sodium fluoride/potassium oxalate tubes, and the plasma should be separated and stored at -20°C until it can be analyzed. Blood for pyruvate analysis should be mixed at a 1:1 ratio with 8% perchloric acid and centrifuged. The supernatant should be removed and kept frozen and analyzed within 30 days. Delays of more than 1 hour or more before deproteinization of samples can induce major elevations of the lactate/pyruvate (L/P) ratios. Evaluation of plasma lactate, pyruvate, and their molar (L/P) ratio can provide important information in the evaluation of possible metabolic myopathies and is critical for the diagnosis of mitochondrial diseases. Lactate and pyruvate readily diffuse from working muscles, and levels of these metabolites in venous blood can be used to monitor the integrity and level of activation of the energy pathways from which they arise. Although not fully characterized in veterinary medicine, the L/P ratio may provide a preliminary subclassification of a possible metabolic

disorder. The level of these muscle metabolites in venous blood after exercise is also a function of the intensity of work performed. A control animal exercised at a similar relative intensity is necessary for valid comparison. Lactic acidemia is a hallmark of mitochondrial myopathies and has been described in human beings and dogs. When a defect is present in either the pyruvate dehydrogenase complex or the electron transport chain, pyruvate is metabolized to either lactate, by lactate dehydrogenase, or to alanine, by alanine aminotransferase, resulting in lactic acidemia and hyperalaninemia. Documentation of a high serum lactate and pyruvate concentration with a normal L/P ratio is consistent with a defect in pyruvate dehydrogenase or one of the gluconeogenic enzymes. When lactic acidosis is found in association with a high L/P ratio, there is the possibility of an underlying defect of the mitochondrial electron transport chain or pyruvate decarboxylase deficiency. Specific respiratory chain defects giving rise to lactic acidemia can be demonstrated in mitochondrial preparations from cultured skin fibroblasts, but this requires expert laboratory assistance and is not performed on a routine basis (Platt et al. 2004).

Diseases that affect skeletal muscle can also affect cardiac muscle. Cardiomyopathy has been convincingly demonstrated in muscular dystrophies (MD), mitochondrial myopathies, and, most recently, in a dog with severe necrotizing myopathy and in horses with atypical myopathy. Troponin is located primarily in myofibrils and is the regulatory protein of contractile skeletal and cardiac muscle, regulating the interactions of actin and myosin in muscle contraction. Troponin is composed of 3 subunits: troponin C, the calcium binding subunit; troponin I, the inhibitory component; and troponin T, the tropomyosin-

binding subunit. Genes distinct from those encoding skeletal muscle isoforms encode cardiac troponin I, and this troponin is a marker of myocardial cellular injury. Thus, cardiac troponins are more specific for cardiac damage, compared with the specificity of LDH or CK activity or myoglobin concentration. Furthermore, cardiac troponin concentrations are generally not affected by nonspecific types of skeletal muscle damage, including trauma, IM injections, or strenuous exercise. Following myocardial damage, troponin I increases in 3–6 hours, peaks at 14–20 hours, and returns to normal in 5–7 days. Cardiac troponin I has largely replaced CK isoenzyme testing in people and may be of value for assessing myocardial damage in animals. The value of other possible new biomarkers, such as skeletal muscle troponin and fatty-acid-binding protein-3, in assessing muscle damage has not been fully evaluated in neuromuscular diseases affecting people and companion animals (Shelton 2010).

Urinary organic acids can be quantified by gas chromatography–mass spectrometry. Organic acidurias are most commonly genetic inborn metabolic errors resulting in accumulation of non-metabolizable organic acids in tissues and biologic fluids. Numerous disorders causing these abnormalities have been described in human beings, and, recently, there have been several such disorders causing neurologic and neuromuscular clinical signs documented in dogs. The accumulating organic acids are excreted in urine in free or esterified forms. Analysis of the profile of the abnormal excreted compounds provides a basis for the diagnosis of the genetic disorder underlying the acidotic syndrome. Mitochondrial defects of β -oxidation can also result in increased urinary excretion of organic acids. Plasma amino acids can be quantified by automated column

chromatography. The analysis of several amino acid profiles can be useful in determining the cause of muscle metabolism disorders. These include alanine and hypoxanthine, which readily diffuse from working muscle into the venous blood as markers of the integrity and level of activation of the energy pathways from which they arise. These tests are only available at a few laboratories (Platt et al. 2004).

Muscle damage will result in the release of muscle enzymes and the muscle protein myoglobin into the blood (measured in the serum or plasma) and myoglobin is rapidly filtered by the kidneys and appears in the urine (making it appear dark red) (Moses et al. 2007). Myoglobinuria refers to excessive myoglobin in the urine, resulting in brown-colored urine, and indicates an abnormal pathophysiologic state usually in association with severe muscle damage and clinical signs of weakness and myalgia. Myoglobinuria is frequently associated with rhabdomyolysis, a severe form of myonecrosis. Recurrent myoglobinuria may be found in inherited metabolic myopathies, such as glycogen storage disorders, fatty acid oxidation disorders, and mitochondrial myopathies, and in MD. Myoglobinuria may also occur as an isolated event following trauma, exposure to drugs and toxins, infectious diseases, fever, and excessive work, and malignant hyperthermia syndrome. Myoglobinuria is one of the most frequent causes of pigment nephropathy and acute kidney injury. Brown urine may indicate $>250 \mu\text{g/mL}$ myoglobin in urine. In general, urine myoglobin concentration is not more sensitive for the detection of neuromuscular disease than serum CK activity, and no clear correlation exists between the serum CK activity and urine myoglobin concentration (Shelton 2010).

Urine, plasma, and muscle concentrations of total, free, and esterified carnitine can be determined by radioisotopic enzyme assay. A complex metabolic equilibrium exists for the various carnitine and acylcarnitine fractions in the different body compartments, between tissue and extracellular fluid pools, and for the fractions of these pools excreted in urine. Therefore, analysis of carnitine metabolism requires the determination of free and esterified carnitine concentrations in plasma, urine, and muscle. The most complete information on carnitine status is gained from evaluation of total, free, and esterified forms of carnitine in all three compartments. Because muscle is the largest reservoir of carnitine in the body, it is muscle carnitine that binds to abnormal organic acids, with ultimate excretion in the urine. Thus, carnitine may be depleted in muscle but be increased or normal in the plasma. Evaluation of the complete carnitine status is necessary for rational therapeutics of primary or secondary disorders of carnitine metabolism (Platt et al. 2004).

ELECTROMYOGRAPHY

By definition, electromyography (EMG) is the recording and study of insertional, spontaneous, and voluntary electric activity of muscle. In human beings, EMG is routinely performed on the awoken patient, where voluntary muscle contraction can be used to assess recruitment of additional motor units and interference patterns with increasing strength of muscle contraction. Because of the difficulty of patient compliance, awoken EMG is not routinely performed in small animals. Instead, most patients are evaluated under general anesthesia, where insertional activity and spontaneous muscle activity are primarily assessed (van Nes 1986; Cuddon 2002).

All EMG testing is subject to technical difficulties because of external noise from other electric outlets (60-cycle interference) or anesthesia related equipment in the same room and ideally should be performed in a Faraday room. The animal should be placed on a padded surface, grounded to the machine and in a warm environment to prevent artifactual changes in results. Four major types of EMG recording electrodes are available. The two most commonly used in veterinary medicine are the concentric needle electrode and the monopolar needle electrode. The concentric (coaxial) needle electrode measures the potential difference between a nichrome, silver, or platinum wire and the stainless-steel shaft that surrounds it. Monopolar needle electrodes are composed of insulated stainless steel, except at the tip. Each of these types of recording electrodes has its advantages and disadvantages. The concentric needle electrode detects less background noise than monopolar needles, and it detects fibrillation potentials more often, because these potentials are most commonly induced by actual needle

insertion rather than occurring spontaneously. The fact that concentric needle electrodes cause more tissue damage than monopolar electrodes allows the detection of fibrillation potentials more easily. Monopolar electrodes are less electrically stable and noisier than concentric needle electrodes, although they do produce less pain and record a considerably larger potential from the same source than the concentric needle. The monopolar electrodes are also able to detect distant potentials as a result of their substantial interelectrode distance. Although surface electrodes are used in human beings, they are not suitable for veterinary use because of technical difficulties related to poor electrode–skin contact as a result of the presence of a hair coat. Bipolar concentric needle electrodes, the fourth electrode type, have been rarely used in veterinary medicine. No matter which electrode type is used, the sensitivity of the EMG examination is increased dramatically by increasing the number of evaluation sites (or passes) in each muscle and also by increasing the number of muscles examined. Abnormal spontaneous muscle activity is commonly a patchy phenomenon with considerable variation in location. Normal resting muscle is generally electrically silent.

Four types of electric activity, however, may be seen in normal muscle: insertional activity, miniature end-plate potentials (MEPPs), end-plate spikes, and motor unit action potentials (MUAPs). Insertional activity can be used to determine muscle excitability. Normal insertion activity is produced by mechanical damage to the myofibers as a result of the action of needle placement, causing brief spontaneous bursts of electric activity that begin and end abruptly as the needle is placed (Fig. 1A). They consist of positive or negative high-frequency

spikes in a cluster. This produces a crisp static sound. In normal muscle, this activity should last a few hundred milliseconds depending on the speed and magnitude of electrode movement, with no indication of waxing and waning. It can be prolonged in disease states that cause instability of the muscle membrane such as denervation or inflammation and can be decreased in severe muscle atrophy or fibrosis caused by a decreased number of available myofibers. MEPPs represent low-amplitude electric activity in normal muscle caused by normal sustained random spontaneous release of single quanta of acetylcholine (ACh), resulting in depolarization at the postsynaptic membrane (see Fig. 1*B*). MEPPs are similar to the sound heard when listening inside a seashell. They usually have 5 to 15 μV amplitude, although they can be as high as 50 μV . They have 1 to 2 milliseconds duration. Because they do not result in depolarization of the entire myofiber, they are only seen if the recording electrode is placed in close proximity to an end plate. Diseases that decrease ACh release from the presynaptic membrane such as botulism produce MEPPs with normal amplitude but decreased frequency. Conversely, diseases such as myasthenia gravis that decrease binding of ACh postsynaptically cause decreased amplitude but normal frequency of MEPPs. Severe denervation results in a complete absence of MEPPs. A waveform that is commonly associated with MEPPs is the end-plate spike, which results from discharge of a single muscle fiber that is excited by activity in nerve terminals. These spikes occur intermittently, with an irregular firing rate of 5 to 50 Hz and with an amplitude ranging from 100 to 300 μV .³¹ They have an initial negative (upward) deflection from baseline and are usually biphasic (Fig. 1*B*). Their appearance is similar to that of fibrillation potentials. MUAPs are seen only

with mild voluntary muscle contraction and represent isolated discharges from one or a few motor units (Fig. 1C). They represent a compound action potential of myofibers in the electrode's recording range. These waveforms are generally biphasic or triphasic, with an initial negative peak. Amplitudes are variable but are usually in the range of 100 μ V to 3 mV and fire at a rate of 5 to 7 Hz. They increase in frequency with increased activation of the same and new motor units, that is, with increasing strength of contraction (commonly termed *recruitment*). With maximum voluntary muscle contraction, there is a simultaneous discharge of many different MUAPs, precluding individual MUAP recognition (termed *interference pattern*). Patterns of MUAPs during maximal contraction are affected differently in various disease states. Myopathy produces a decrease in MUAP amplitudes but with normal density, whereas partial denervation results in a decreased density of MUAPs. The presence of many giant MUAPs (500 μ V–5 mV) would indicate reinnervation. These giant MUAPs are commonly polyphasic, because the new collateral nerve branches have thinner myelin sheaths than the established axons and thus have slower conduction velocities. As a result of poor patient compliance, evaluation of MUAPs and recruitment patterns is not used extensively in veterinary medicine. One area of application, however, is in the assessment of the severity and extent of peripheral nerve injury after trauma. The absence of any inducible MUAPs in a specific muscle with weight bearing (extensor muscle) or limb flexion (flexor muscle) would indicate severe nerve injury.

There are four patterns of abnormal spontaneous activity in EMG: fibrillation potentials, positive sharp waves, complex repetitive discharges (CRDs), and myotonic potentials.

Fibrillation potentials and positive sharp waves represent the same underlying pathologic changes and differ in morphologic findings only in relation to their orientation to the recording electrode at the time of discharge. Both arise from spontaneously firing, hypersensitive, single myofibers as a result of destabilization of the sarcolemmal membrane. This can occur in denervation, polymyositis, muscular dystrophy, or other myopathies. Fibrillation potentials consist of biphasic or triphasic waves with the initial deflection usually in the positive (downward) direction, except if recorded within an end-plate region (Fig. 2). These waves are 10 to 200 μ V in amplitude and usually appear in bursts. They have the sound of frying eggs or the wrinkling of tissue paper.

Positive sharp waves consist of an initial positive spike followed by a much shorter slow negative potential (Fig. 3A). These waveforms have a variable amplitude (50 μ V–4 mV) and a much deeper sound than fibrillation potentials. They also represent irritated myofiber membrane, although unlike fibrillations, the potential stops at an area immediately adjacent to the recording electrode.

CRDs are polyphasic repetitive waveforms with a uniform frequency, shape, and amplitude and represent spontaneous discharge of multiple myofibers firing in near synchrony (Fig. 3B and 3C). These trains of waves, with amplitudes in the range of 100 μ V to 1 mV, can begin spontaneously or after needle movement and always have an abrupt onset and cessation. CRDs are more often associated with chronic denervation, although they are also seen in some myopathies such as that

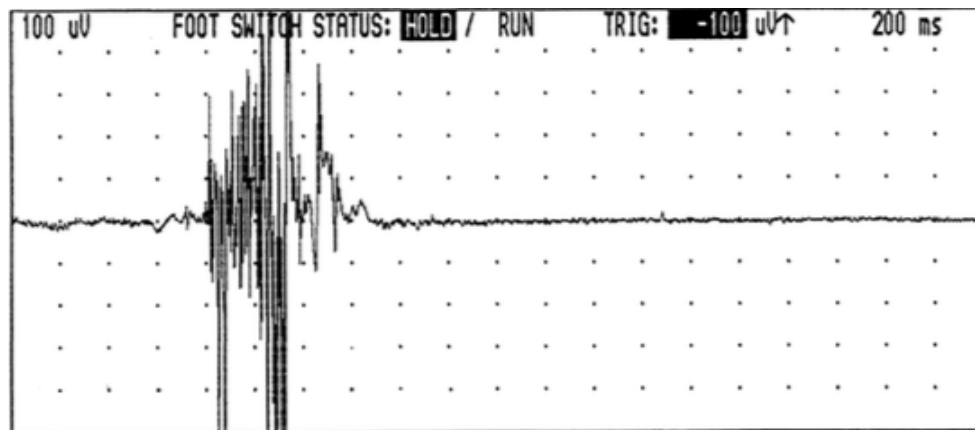
associated with hyperadrenocorticism. These discharges are commonly mistaken for myotonic potentials.

Myotonic potentials are the final abnormal spontaneous activity seen on EMG. These are repetitive discharges occurring at a rate of between 20 and 80 Hz, which must wax and wane over time. They represent independent repetitive discharges from single injured myofibers. The individual waveforms can be one of two types — either a sustained run of positive waves resembling positive sharp waves (PSWs) or a sustained run of biphasic spike potentials (an initial small positive peak followed by a larger negative peak) resembling fibrillation potentials. The waxing and waning nature of the frequency and amplitude of these waveforms produces the characteristic “dive-bomber” sound. This discharge is characteristic of myotonia congenita. There are a number of misconceptions concerning the amount of information that EMG alone can reveal about the extent and etiology of neuromuscular disease. EMG has limited value in:

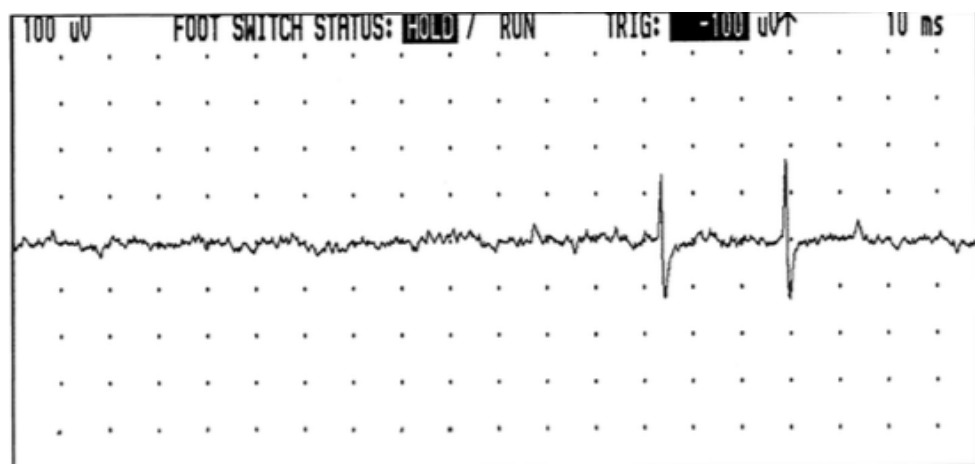
- (1) Inferring clinical symptoms and neuropathic deficit,
- (2) Distinguishing between neuropathy and myopathy,
- (3) Inferring involvement of small-diameter fibers,
- (4) Inferring underlying biochemical or other pathophysiologic derangement,
- (5) Inferring the presence and type of pathologic alterations in single fibers and Schwann cells.

Despite these limitations, EMG is clinically useful in differentiating between denervation atrophy and disuse muscle atrophy. This distinction may not always be evident historically or on physical examination, and this knowledge almost definitely alters therapy. EMG also has a high degree of sensitivity in detecting

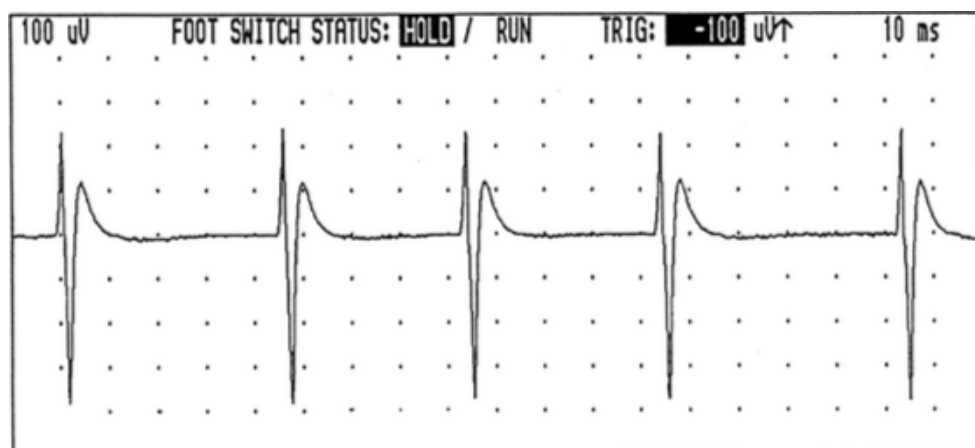
peripheral nerve axonal loss, being able to detect abnormalities even when as few as 5% of the total number of axons in the nerve have been affected. It is important to remember, however, that any changes associated with denervation are not detected in dogs and cats for a minimum of 4 to 5 days after the initiating insult has occurred and that maximal changes are not seen until day 8 to 10. The first abnormality that is detected is an increase in insertional activity, followed closely by the presence of fibrillation potentials. Another factor that influences the delay time of fibrillations after injury is the distance from the injury site to the muscle, i.e. the shorter is the distance, the earlier the fibrillations appear. A significant decrease in the intensity of spontaneous activity over time is most commonly indicative of successful reinnervation; however, this phenomenon also can be seen with end-stage fibrosis. Mean values for serum CK in the dog do show increases after electrophysiologic evaluation, although they are still usually within the normal range. Dogs seem to be less sensitive to sarcolemmal damage from EMG electrodes compared with people. There is a delay, however, in this increase, with CK measurements immediately after EMG being unaffected. These CK values also return to normal by 48 hours after EMG examination (Cuddon 2002).



A

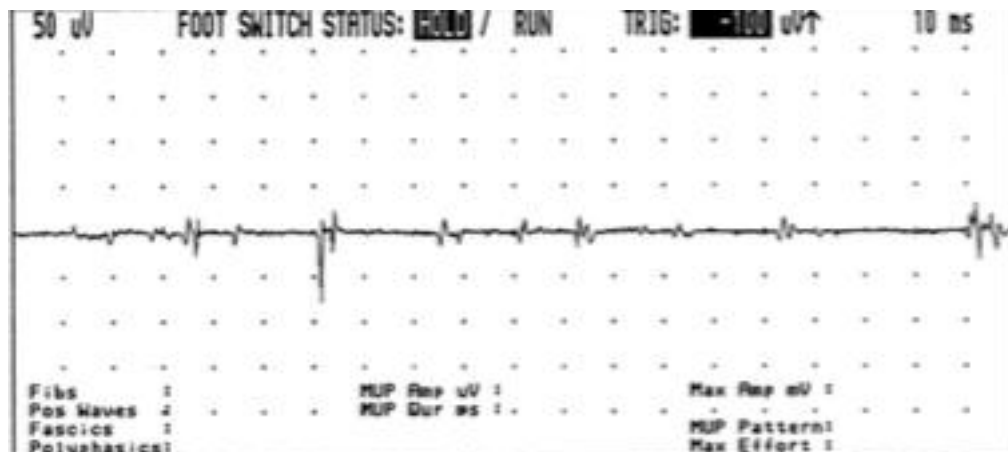


B

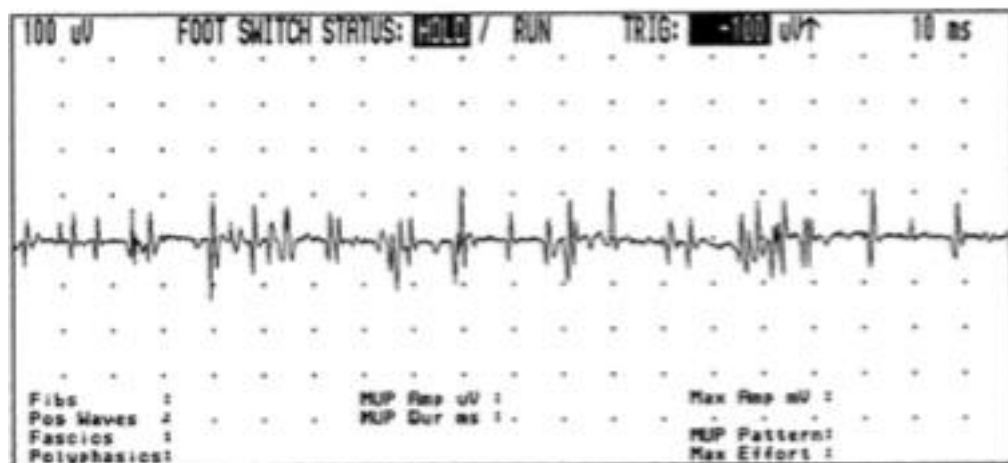


C

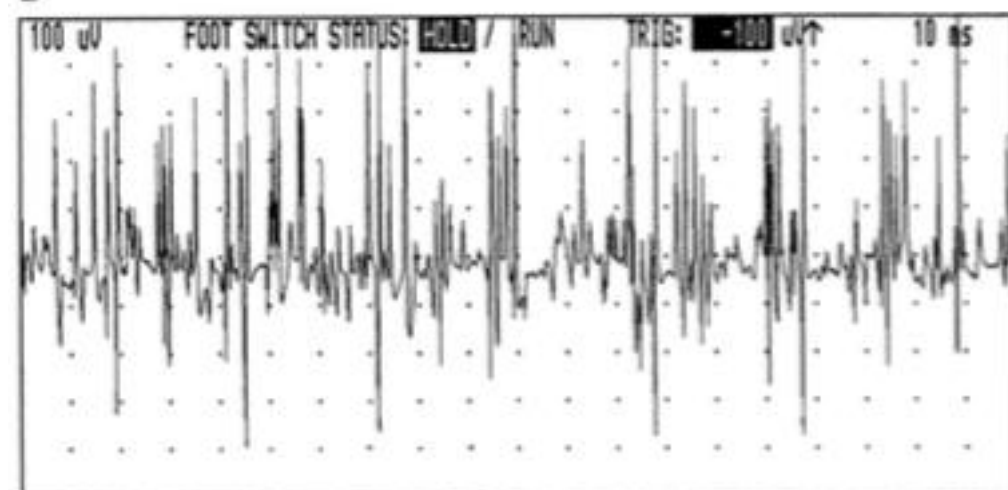
Figure 1 Types of electrical activity seen in normal muscle during EMG evaluation: A Insertional activity; B Miniature end-plate potentials with two end-plate spikes indicating close proximity of the needle to an end-plate; C Motor unit action potentials seen during voluntary muscle activity in an awake animal. (Modified by Cuddon, 2002)



A

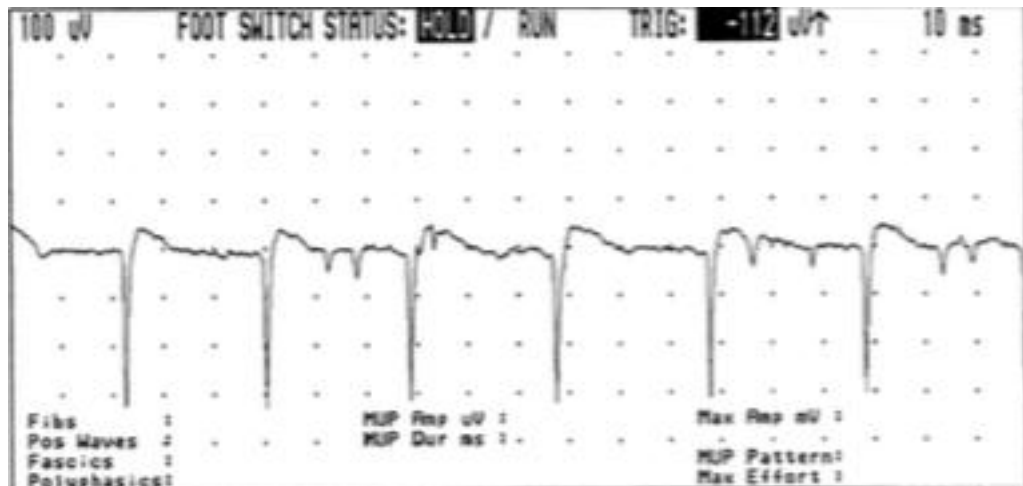


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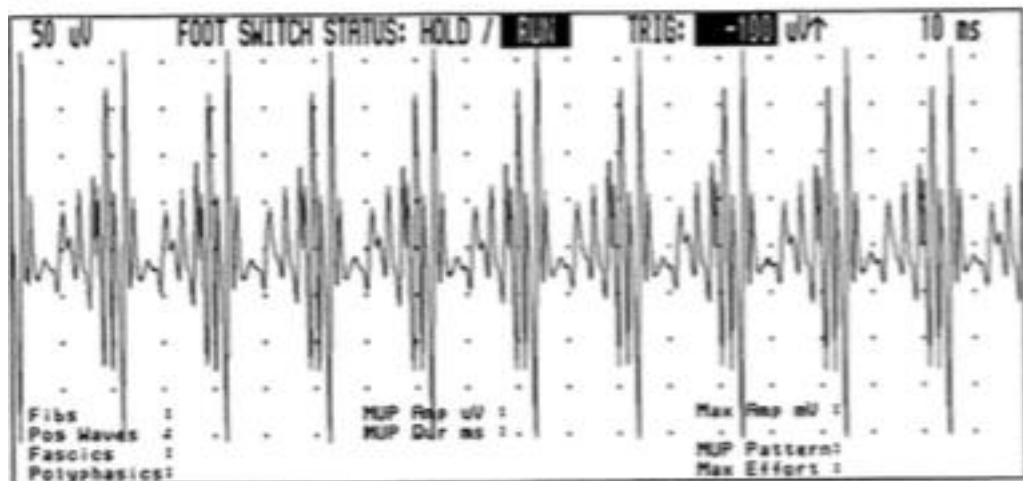


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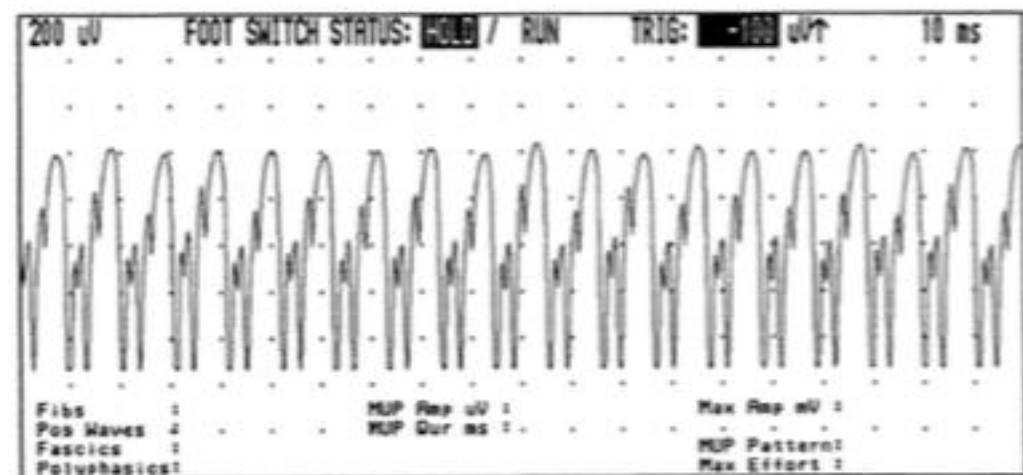
Figure 2 Abnormal spontaneous electrical activity, in the form of fibrillation potentials, observed in muscle during EMG evaluation. **A**, Mild density of fibrillation potentials (50 V/div; 10 msec/div). **B**, Moderate density of fibrillation potentials (100 V/div; 10 msec/div). **C**, Severe fibrillation potentials (100 V/div; 10 msec/div). (Modified by Cuddon, 2002)



A



B



C

Figure 3 Additional abnormal spontaneous electrical activity observed during EMG evaluation: A, Positive sharp waves (100 V/div; 10 msec/div). B, Complex repetitive discharges (50 V/div; 0 msec/div). C, Complex repetitive discharges (200 V/div; 10 msec/div). (Modified by Cuddon, 2002)

MUSCLE BIOPSY

Biopsies of muscle, and in some cases peripheral nerve, should be collected early in the course of diagnostic evaluation of an animal with a neuromuscular disease, rather than waiting until extensive muscle damage, fiber loss, and fibrosis have occurred, when the chances for a successful treatment are diminished. Delay in diagnosis and initiation of appropriate therapy may result in irreversible fibrosis and limb contractures (Shelton 2010).

Muscle biopsies should be collected by an open surgical procedure allowing visualization of the orientation of muscle fibers. Evaluation of muscle biopsy specimens often offers the most specific information required to make a diagnosis. In cases with diffuse involvement, it is better to biopsy easily accessible muscles such as the *vastus lateralis* or *biceps femoris* muscles. Obviously, in animals with focal myopathies, the involved muscles must be biopsied. Clinicians are strongly advised to contact their reference laboratory before performing the biopsy in order to be clearly informed about the precise requirements regarding sampling, handling and shipping of the specimen. Generally, fresh or flash frozen samples are easiest to analyze if they have been prepared and shipped properly. All histochemical, histological and immunohistochemical investigations can be performed on such samples. Fixation in formaldehyde does not allow a comprehensive analysis of the samples. Routine analysis of muscle samples should include histochemical stains such as the ATPase stain to evaluate the presence and distribution of the different muscle fiber types. For feline muscle, the ATPase stain at pH 4.45 allows good differentiation between the main fiber types I, IIA, and IIB. The classical stains

haematoxylin and eosin (HE) or Gomori trichrome (GT) are used to screen the specimen with respect to muscle fiber size variations, presence of central myonuclei, fiber degeneration and necrosis, regenerative attempts, or infiltrates with inflammatory cells or connective tissue. Specific techniques aimed at staining fibrous tissue, fatty infiltrates and other lesions can then be used as needed (Gaschen et al. 2004).

IMAGING STUDIES

Magnetic resonance imaging (MRI) can be used to identify areas of active inflammation seen as high-signal intensity areas on fluid-sensitive sequences, and minimal atrophy. Using imaging to target the regions of concern could improve the efficacy and cost-effectiveness of biopsy. Moreover, MRI is useful to define some patterns of muscle atrophy, which can help in the diagnosis of other myopathies such as muscle dystrophies (Milisenda et al. 2014).

INFLAMMATORY MYOPATHIES

Myopathies may be classified as inflammatory or noninflammatory. Inflammatory myopathies (IMs) include generalized diseases, such as polymyositis and dermatomyositis, and focal IMs, such as extraocular and masticatory muscle myositis. (Shelton 2006)

The inflammatory myopathies are infectious or immune mediated. Infectious myositis has been reported in association with protozoal organisms (toxoplasmosis, neosporosis, hepatozoonosis), bacterial infections (leptospirosis), and migrating parasites. (Taylor 2000)

Myositis is inflammation of muscle, when several muscles are affected, the term polymyositis is used to describe the condition. The signs are muscle weakness, with muscle swelling and pain during the acute stages and muscle atrophy during the chronic stages. Because the inflammation often affects the muscle cell membrane, muscle intracellular enzyme may leak out and cause serum activity measurements to be higher than normal. Myositis may be of infectious or noninfectious origin. Infectious causes may be bacterial, often *Leptospira* spp. and *clostridia* spp., but also parasitic, such as *Toxoplasma* spp. and *Trichinella* spp. Non infectious causes of myositis in small animals are probably immunomediated conditions. (Amann 1987).

INFLAMMATORY MYOSITIS

– MASTICATORY MYOSITIS:

Masticatory muscle myositis (MMM) is an inflammatory disorder selectively involving the muscle of mastication (temporalis, masseter, medial and lateral pterygoid, and rostral portions of the *digastricus* muscle) in the dog. (Amann 1987; Shelton 2007, Taylor 2000)

Two separate disorders were described initially (eosinophilic myositis and atrophic myositis), but it is probable that the two are different phases of a single disease process. (Taylor 2000)

Although these names suggest a different pathogenesis, they likely represent the acute and chronic phases of masticatory muscle myositis. The acute phase is characterized clinically by jaw pain, trismus, and swelling, and the chronic phase is characterized by marked muscle atrophy. Without early recognition and aggressive treatment, myofiber loss and muscle fibrosis may result in irreversible jaw dysfunction and severe muscle atrophy. (Melmed et al. 2004)

This is an autoimmune disease in which B-lymphocyte-mediated antibodies are directed against type 2 M fibers in masticatory muscles. Type 2 M fibers are the dominant fiber type in masticatory muscles that are not present in limb muscles (Braund 2003). In dogs with MMM, necrosis and phagocytosis are limited to these fibers, and there is circulating IgG directed against the unique myosin component of these fibers. (Taylor 2000)

It remains unknown what initiates formation of autoantibodies or why they are directed specifically against type 2M fibers. Some theories suggest that molecular mimicry may play a role, with antibodies or T cells generated in response to an

infectious agent that subsequently cross reacts with self antigens. In this scenario, bacterial antigens would have a similar peptide sequence or conformational structure to some component of the 2M myofibers. Antibodies directed against these bacterial antigens could potentially cross-react with these myofibers. (Melmed et al. 2004)

The classical clinical presentation for masticatory muscle myositis is inability to open the jaw (trismus), jaw pain, and swelling or atrophy of the muscles of mastication. The average age of onset for masticatory muscle myositis is 3 years of age, although patients have reportedly been as young as 4 months of age. The disease can occur in any breed, but there may be a predilection for large-breed dogs, with overrepresented breeds including German shepherds, Labrador retrievers, Doberman pinschers, and golden retrievers. Cavalier King Charles spaniels appear to have a genetic predisposition to masticatory muscle myositis. No gender predilection has been found.

In acute form of MMM, there is recurrent painful swelling of the muscle of mastication. Exophthalmos and prolapsed of the third eyelid may occur, caused by pressure on retrobulbar tissues from swollen musculature. Rarely enough stretching or compression of the optic nerve occurs to cause blindness. Pyrexia, submandibular and prescapular lymphadenopathy, and tonsillitis are variably present during recurrent symptomatic episodes, which may last 1 to 3 weeks. Affected dogs are reluctant to eat during episodes and may salivate profusely. Palpations of the muscles of the head and attempts to open the mouth are met with resistance because of pain. (Braund 2003, Melmed et al. 2004, Podell 2002, Taylor 2000)

Unfortunately, most owners do not recognize a problem until the chronic phase, which is characterized by marked muscle atrophy with or without persistent trismus. Enophthalmos may be present in the chronic phase because of atrophied pterygoid muscles. (Melmed et al. 2004)

Chronic MMM is more commonly recognized than the acute form. Affected dogs are bright, alert, and systemically normal, but there is progressive severe atrophy of the temporalis and masseter muscles, resulting in a skull-like appearance of the head. Dogs with chronic MMM may have difficulty in opening their mouths wide enough to eat; in some cases, the jaw cannot be opened even under general anesthesia. (Taylor 2000)

The diagnosis of MMM is suspected based on clinical findings. Initial diagnostic testing should include a complete blood count and serum chemistry profile, including a creatine kinase (CK) level. Biochemical changes that have been documented in patients with masticatory muscle myositis include hyperglobulinemia, mild anemia, and proteinuria. Although peripheral eosinophilia has been reported, it has not been a consistent clinicopathologic finding. CK levels are frequently elevated during the acute phase, but are often normal as the disease becomes more chronic. The degree of enzyme elevation, if present, is relatively less than that in patients with polymyositis, because of the smaller muscle mass affected.

A confirmatory blood test for circulating antibodies against masticatory muscle is critical to confirm the diagnosis. Clinical signs compatible with masticatory muscle myositis and positive results from a 2M antibody test confirm the diagnosis. However, false negatives may occur if immunosuppressive dosages of

corticosteroids have been administered for 7 to 10 days before testing and in end-stage masticatory muscle myositis with loss of myofibers and fibrosis. (Melmed et al. 2004, Podell 2002, Taylor 2000)

Other procedures that may aid in diagnosing masticatory muscle myositis include radiology and advanced imaging, electrodiagnostics, and histologic evaluation of biopsy specimens. (Melmed et al. 2004)

Electromyography (EMG) may be a useful diagnostic procedure, particularly in differentiating masticatory muscle myositis from polymyositis. Electromyographic abnormalities seen with myopathic disease include increased insertional activity, fibrillation potentials, positive sharp waves, and complex repetitive discharges. Abnormalities may be severe during the acute phase of the disease. However, EMG results may be normal in patients with end-stage disease because of severe atrophy or loss of muscle fibers and fibrosis. In these patients, the only change evident may be decreased insertional activity due to loss of muscle fibers.

Evaluating a muscle biopsy can also provide diagnostic confirmation of the disease as well as additional information regarding prognosis, particularly when muscle atrophy is present and significant fibrosis is suspected. Muscle biopsy documents the severity of fiber loss and degree of fibrosis, which are important in determining the long-term prognosis and probable success of therapy. Muscle biopsy is a simple surgical procedure. Biopsies are typically obtained from the temporalis muscle; however, care must be taken to avoid sampling the frontalis muscle, which overlies the temporalis muscle, because it is not affected in masticatory muscle myositis. In addition, biopsies are important for prognosis and

determining the usefulness of immunosuppression. If only fibrosis is present without remaining myofibers or inflammation, the rationale for using immunosuppression should be questioned. (Melmed et al. 2004, Taylor 2000)

A favorable outcome in masticatory muscle myositis necessitates early accurate diagnosis and appropriate therapy. Treatment is centered on aggressive immunosuppression, which is generally achieved by corticosteroid administration. The cornerstone of therapy is prednisone at 2 mg/kg PO bid during the acute phase. This dose should be maintained until maximum jaw function has been regained and CK levels have returned to normal. At that time, prednisone can be slowly tapered to the lowest every-other-day dose that abates clinical signs. This process should generally occur slowly over 4 to 6 months. Although low-dose alternate-day therapy is generally well tolerated, long-term prednisone may result in iatrogenic hyperadrenocorticism and susceptibility to infections. Owners should be prepared for resultant polyuria, polydipsia, and polyphagia associated with prednisone administration as well as the potential for steroid-induced gastric ulcers. In addition, corticosteroid therapy alone can result in masticatory muscle atrophy. If the side effects of prednisone therapy cannot be tolerated, alternative immunosuppressive agents may be used. Azathioprine is another immunosuppressive drug that can be considered in addition to traditional corticosteroid therapy. Although azathioprine is generally not included in the initial therapy for masticatory muscle myositis, it can be used in conjunction with prednisone in patients that are unable to tolerate the side effects of corticosteroids or are refractory to prednisone therapy alone. Azathioprine should be dosed at 2 mg/kg PO q24–48h and continued over several months while prednisone is slowly

tapered to a maintenance dose. Side effects associated with azathioprine include bone marrow suppression and hepatotoxicity. If untreated or treated inappropriately, the acute phase will progress to the chronic phase. A common problem in treating masticatory muscle myositis is using an inadequate dose of corticosteroids for too short a time. It is common for masticatory muscle myositis to respond initially to therapy, but relapses usually occur quickly if treatment is discontinued prematurely. The chronic phase is marked by severe muscle atrophy resulting from gradual replacement of muscle fibers with fibrous tissue. Corticosteroids may prove helpful in the chronic phase, although lower doses are recommended. The clinical application of corticosteroids in the chronic phase is based on the belief that therapy may reduce further fibrosis. Patients experiencing significant trismus may require gruel diets to maintain adequate nutritional intake. Patients can also be encouraged to chew toys or bones to promote use of their masticatory muscles. The literature has historically recommended forcible opening of the jaw while patients are under anesthesia. But nowadays retraction of the jaw is strictly contraindicated. The prognosis is determined by the degree of fibrosis present and the clinical response to immunosuppression. Aggressive treatment during the acute phase generally results in a good prognosis. It is important to remember that corticosteroids alone can cause muscle atrophy and, therefore, progressive atrophy may not be indicative of worsening disease. Treatment failure and relapses usually result from inadequate immunosuppression and hasty discontinuation of corticosteroids. Patients treated in the chronic phase of the disease carry a more uncertain prognosis but can do well if extensive

fibrosis does not result in persistent jaw dysfunction. (Melmed et al. 2004, Podell 2002, Taylor 2000)

– EXTRAOCULAR MUSCLE MYOSITIS

Extraocular polymyositis (EOM) is a relatively uncommon condition of dogs, of which there are limited reports in veterinary literature. (Mitchell 2008) EOM is a focal inflammatory myopathy of dogs localized to the extraocular muscles. Masticatory and limb muscles are normal, suggesting that myofiber-specific antigens unique to the extraocular muscles may play a role in their selective immune-mediated destruction. (Taylor 2000)

This disorder has been reported primarily in young dogs aged 6 to 24 months. Large-breed dogs, particularly Golden Retrievers, are most commonly affected, and females are most frequently affected. (Taylor 2000, Mitchell 2008) Acute bilateral exophthalmos is the most dramatic finding. (Taylor 2000) EOM can cause exophthalmos and strabismus due to inflammation of the extraocular muscles. EOM is typically non-painful, and protrusion of the third eyelid is not a feature. (Mitchell 2008) Serum CK is normal or mildly increased. Orbital sonography reveals swollen extraocular muscles and rules out a retrobulbar mass or abscess. The extraocular muscles are smaller and difficult to biopsy due to their location. Muscle lesions are confined to these muscles: in typical muscle sections affected with EOM, histopathology shows myonecrosis with mononuclear infiltrate of CD3+ T-lymphocytes and occasional macrophages in extraocular muscle bellies. The inflammatory nature of the affected extraocular muscles and excellent responsiveness to steroids suggests an immune-mediated basis for the myositis.

Swelling of the extraocular muscles restricts globe movement resulting in strabismus. (Mitchell 2008)

Treatment consist in using immunosuppressive dose of corticosteroids, which can be gradually tapered after three weeks, depending upon response. Azathioprine can be used when the corticosteroids are contraindicated or unsuitable for the patient. Early treatment carries a good prognosis and there may be no permanent effects. Prolonged swelling of affected muscles may result in fibrosis leading to enophthalmos and pronounced strabismus which could hinder vision. (Taylor 2000, Mitchell 2008)

– IDIOPATHIC POLYMYOSITIS

In human polymyositis (PM) is classified as a separate entity among idiopathic inflammatory myopathies, but it is considered as the least common since it is an exclusion diagnosis. It is believed that physical, chemical or external infectious agents act upon a genetically predisposed person. The relationship of PM with other autoimmune disorders, the existence of autoantibodies, histocompatibility genes, the presence of T cells in muscle tissue and their response to immunotherapies have led PM to be considered an autoimmune disease, but no specific target antigens have been identified yet. (Milisenda et al. 2013)

Idiopathic polymyositis is an inflammatory myopathy in the dog and cat not associated with any other systemic connective tissue disease or infectious cause. The condition can either affect focal muscle groups (extraocular, laryngeal) or manifest as multifocal or diffuse involvement of skeletal muscle. (Podell 2002)

In the dog, there seems to be a predilection for larger breed and mature to older dogs, although any age or breed of dog may be affected. Large-breed adult dogs are most commonly affected, with many reported cases in German Shepherds. There may be a slight gender predilection for female dogs. (Taylor 2000)

The clinical signs are variable and may wax and wane initially. Progressive exercise intolerance with acute exacerbation of weakness may occur. The gait is often characterized by a profound stiffness, with dogs looking as if they are gingerly tip-toeing with extremely short steps (“walking on eggshells”). Cervical ventroflexion and a lordotic posture can be seen. (Podell 2002)

Muscle palpation elicits obvious pain in some dogs with PM, although non-painful muscle atrophy occurs in others. Regurgitation caused by megaesophagus, dysphagia, excessive salivation, pyrexia, and a weak bark are occasionally recognized. Signs may be intermittent. (Taylor 2000)

The criteria for a definitive diagnosis of idiopathic polymyositis are not well defined in veterinary medicine. Podell diagnoses idiopathic polymyositis by confirming three or more of the following: (1) clinical signs (as described), (2) elevation of CK, (3) abnormal EMG with a normal motor nerve conduction study, (4) negative autoimmune and infectious disease antibody titers, and (5) inflammatory muscle biopsy.

Thoracic radiographs are recommended to evaluate for the presence of megaesophagus even if it is not clinically evident. Whenever possible, EMG should be performed to document that abnormalities are present in multiple muscle and to identify the most dramatically involved muscle groups prior to biopsy. When a severely affected muscle is located, it is recommended that a

biopsy be taken of that same muscle group on the opposite side of the dog to avoid artifacts caused by the EMG needle. EMG changes can include prolonged insertional activity, positive sharp waves, fibrillation potentials, and bizarre high-frequency discharges. (Taylor 2000)

If EMG is not available, the most painful muscles should be biopsied before the onset of any therapy. Typical changes in the muscle include a mononuclear inflammatory infiltrate with or without eosinophils that is often perivascular, invasion of non-necrotic fibers by cellular infiltrates, and myofiber necrosis. (Podell 2002)

Therapy is focused on immunosuppression, initial pain relief, and supportive care. Prednisone at 2 mg/kg administered orally twice daily is the recommended initial therapy. Care must be taken if aspiration pneumonia is present when instituting immunosuppressive therapy. It is advisable to begin systemic appropriate bactericidal antibiotics for 24 hours before the onset of corticosteroid therapy in the face of aspiration pneumonia. Prednisone can then be gradually increased over several days while the animal is monitored closely. A fentanyl patch is recommended for pain relief for the first 72 hours. Appropriate changes in feeding should be implemented if megaesophagus is present. The prognosis for idiopathic polymyositis is generally good in dogs and cats without megaesophagus. Long-term, and sometimes lifelong, corticosteroid therapy may be needed to prevent clinical relapse. Alternative immunosuppressive therapy may be helpful to reduce the reliance on higher doses of corticosteroids. (Podell 2002) Whenever the diagnosis of an inflammatory myopathy is made, every attempt should be made to rule out an infectious cause. Serum titers should be measured against *Toxoplasma*

gondii, *Neospora caninum*, and tick-related diseases when appropriate. Clinical signs of infectious polymyositis may be present as early as 4 weeks of age and include progressive paraparesis and "bunny hopping" gait with progression to pelvic limb hyperextension and muscle atrophy. Progression to pelvic limb hyperextension is more likely when infection develops prior to 4 months of age. Serum CK concentration is usually elevated. Elevated concentrations of serum and CSF antibodies against *N. caninum* and *T. gondii* support the presence of infection. Occasionally organisms are found within muscle biopsy sections. Treatments have included clindamycin and sulfadiazine and trimethoprim. While some improvement may be noted in neurological function, complete resolution of pelvic limb hyperextension has not been reported to occur. (Shelton 1999)

- FAMILIAL CANINE DERMATOMYOSITIS

Dermatomyositis (DM) or familial canine dermatomyositis is a well documented disease of Collie dogs, of all coat colors and both coat lengths. Dermatomyositis has also been reported in the Shetland Sheepdog (Shelty), Beauceron Shepherd, Pembroke Welsh Corgi, Australian Cattle dog, Lakeland Terrier, Chow Chow, German Shepherd, and Kuvasz. (Braund 2003) Male and female can be affected. DM is an inflammatory disease of striated muscle, skin, and vasculature; in humans, adult and juvenile forms have been described. Perivascular accumulations of inflammatory cells are early changes. Angiopathy is characteristic of this disease, particularly in the childhood form, and a perifascicular pattern of muscle fiber atrophy is characteristic. In dogs, the skin lesions are most problematic, and the muscle lesions usually mild. In contrast, in

humans, the muscle lesions are the most problematic. While the conditions are not identical, vascular lesions are present in both conditions. (Shelton 2007)

An autoimmune pathogenesis with immune complex deposition is suspected, although the definitive target antigens are not identified. According to Podell (2002) the onset of clinical signs is typically within the first 6 months of life, instead according to Braund (2003) the onset is within 2 and 6 months of age, and yet according to Grossa and Kunkle (1987) the onset is usually between the 12 to 14 weeks.

Cutaneous lesions develop mainly on the face and ears, although the tail tip and bony prominence areas may be affected. The initial signs are development of cutaneous vesicles when the dog is between 2 and 4 months of age, followed by erythema, ulceration, alopecia, crusting, and changes in pigmentation. Less commonly, adult dogs may develop more severe dermatologic signs later in life, which may be precipitated by stress events such as trauma, parturition, or even prolonged sunlight exposure. The myositic signs develop typically after the dermatitis and correlate in severity approximate to the degree of dermatitis. The temporalis muscle is the initial and most commonly affected muscle group. Difficulty in prehending food, dysphagia, and temporalis muscle atrophy are classic signs. More severe signs include megaesophagus with regurgitation and generalized polymyositis, leading to diffuse muscle atrophy over time. Unlike idiopathic polymyositis, DM lesions are more prevalent in temporalis and distal appendicular muscles. (Podell 2002)

The cutaneous lesions consist of pustules, ulcers, and vesicles which may progress rapidly to crusted or alopecic areas. Myositis develops several months later and

principally involves muscles of mastication and muscles of the extremities below the elbow and stifle. The muscle lesions appear to correlated with the severity of the skin lesions. Muscle lesions consist of multifocal muscle fiber necrosis, internalization of muscle nuclei, atrophy, fibrosis, and regeneration, and mild to severe interstitial and perivascular inflammatory cell infiltrates (lymphocytes, neutrophils, plasma cells, and macrophages). Small intrafascicular nerves may be surrounded by inflammatory cells. Vasculitis is seen in skin, muscle, and occasionally in other tissues. Necrotizing vasculitis of small venules and arterioles is characterized by fibrinoid thickening of the vessel wall, pyknosis and karyorhexis of endothelial cell nuclei, and neutrophilic inflammation. In many cases, the lesions spontaneously regress by 6 to 8 months of age, although severely affected dogs may have dermatitis throughout their lives. Differential diagnosis of the skin lesions includes demodicosis, dermatophytosis, staphylococcal folliculitis, epidermolysis bullosa simplex, and discoid lupus erythematosus. There is a dramatic increase in serum concentrations of IgG and circulating immune complexes, which may be detected before clinical signs and which show a positive correlation with disease severity, and which decline as animals enter remission. Non-regenerative anemia due to chronic inflammation may occur in severely affected dogs. CK level are usually normal, but may be increased. The presence of fibrillation potentials, positive sharp waves, and bizarre high frequency discharges has been demonstrated electromyographically. (Braund 2003)

The diagnosis of dermatomyositis includes a combination of factors. Five criteria have been used to define human dermatomyositis. These include:

- 1) Symmetrical skeletal muscle weakness
- 2) Muscle biopsy evidence of myositis
- 3) Elevation of serum skeletal muscle enzymes
- 4) Characteristic electromyographic changes
- 5) Dermatologic features consisting of erythematous areas over knuckles, elbows, knees, medial malleoli, face, neck, and upper torso

According to Halgis et al. (1984) the dogs had similar features, including

- 1) Symmetrical muscle atrophy
- 2) Muscle biopsy evidence of multifocal myositis
- 3) Electromyographic changes suggestive of a myopathy
- 4) Dermatologic features of erythema and inflammation of periorbital, facial, and lip skin, and skin surfaces subject to trauma such as elbows, stifles, carpi, tarsi, feet, and sternum.

Therapy revolves around symptomatic relief of skin lesions and immunosuppression with prednisolone, at 1 to 2 mg/kg PO bid. Prolonged or recurrent steroid therapy may be necessary. Additional recommended therapy includes avoidance of sunlight, neutering for female dogs, treatment of underlying pyoderma, and vitamin E. Pentoxifylline, a methylxanthine that increases microvascular blood flow, may have some benefit for treatment of familial canine DM. The overall prognosis for less severely affected dogs is usually good. (Podell 2002; Braund 2003)

MUSCULAR DYSTROPHIES

Muscular dystrophies (MD) are a heterogeneous group of inherited, degenerative, mostly non-inflammatory disorders characterized by progressive muscle weakness and wasting. Until recently, one form of muscular dystrophy was recognized in dogs and cats (the X-linked dystrophin deficiency). More recently, however, merosin (laminin α_2) deficiency and other apparently autosomal forms of muscular dystrophy have been detected. (Shelton & Engvall 2002) A better understanding of the pathogenesis and clinical features of this class of myopathy allows nowadays a progressive enrichment of the literature and the description and molecular characterization of different dystrophies, partially or totally correspondent to analogues human diseases. (McGreevy et al. 2015, Munday et al. 2014; Baroncelli et al. 2014; Atencia-Fernandez S. et al. 2014)

A precise classification of dystrophic muscle disorders is critical since some of the disorders are the result of X-linked inheritance, whereas others are inherited in an autosomal recessive or dominant fashion. An accurate classification is important to animal breeders and owners, because diagnosis, disease progression, prognosis, and patterns of inheritance differ for the various forms of MD. Some forms of MD are severe and lethal relatively early, while other forms are slowly progressive and the animal may stabilize and live a near-normal life, although activity may be limited. (Zucconi E. et al. 2010; Zatz M. et al. 2015) Because of the large number of possible forms of MD, the variable clinical presentations for each one, and the overlap between different forms, it is difficult to classify the disease based solely on clinical signs such as age of onset or pattern of muscle involvement. Furthermore, because limited information is available on MD in dogs and cats

other than for dystrophin deficiency, it is difficult to extrapolate the clinical picture from what is known in the human diseases. A dystrophic myopathy should be considered in any young dog or cat (male or female, mixed breed or purebred) with persistent muscle weakness, muscle atrophy or hypertrophy, gait abnormality, or contractures beginning in the first few months of life. Dysphagia, regurgitation, and dyspnoea may occur as a result of hypertrophy of the lingual, pharyngeal, and oesophageal musculature and the diaphragm. Specific therapies are not currently available and the general prognosis is poor. (Shelton 2004)

The most common form of muscular dystrophy in human beings, Duchenne muscular dystrophy (DMD), was shown in 1987 to be caused by a lack of the muscle-associated protein called dystrophin. A mild form of DMD is often referred to as Becker muscular dystrophy (BMD). Dystrophin is not absent in BMD but is mutated and structurally altered and only partially functional. Several other skeletal muscle proteins have since been identified as involved in other X-linked and autosomal forms of muscular dystrophy. Several of these proteins are directly or indirectly linked to dystrophin. (Shelton & Engvall 2002)

The two most common forms of muscular dystrophy are severe and relatively early lethal. Because of the large number of forms of muscular dystrophy, the variable clinical presentations for each one, and the overlap between different forms, it is difficult to classify disease solely on clinical signs such as age of onset or pattern of muscle involvement. (Shelton et Engvall 2002)

► Muscular Dystrophy in Dogs and Cats with Absence of Dystrophin

DMD associated with an absence of dystrophin and genetic mutations of the dystrophin gene is the most common and best studied of the muscular dystrophies in human beings and companion animals. (Shelton & Engvall 2002) Dystrophin is a 400-kilodalton protein that functions to stabilize the muscle membrane during contraction. (Bergman R.L. et al 2002) The dystrophin gene is located on the X-chromosome; thus, DMD is an X-linked recessive trait transmitted by a female carrier, who is most often asymptomatic and only rarely manifests clinical signs of limb weakness and myopathic changes on electromyography and muscle biopsy. Similar to DMD in human beings, all reported dogs with canine X-linked muscular dystrophy (CXMD) and cats with feline X-linked muscular dystrophy (FXMD) have been male. (Shelton & Engvall 2002) CXMD was first described in the Golden Retriever. (Bergman R.L. et al 2002). Muscular dystrophy with dystrophin deficiency has been documented afterwards in several breeds of dogs. The clinical phenotype of reported dystrophin deficiencies differs between dogs and cats. An absence of dystrophin has been associated in dogs with generally diffuse muscle atrophy, except for specific muscles that hypertrophy (i.e., semimembranosus, semitendinosus, and tongue muscles); on the contrary it is associated with generalized muscle hypertrophy in cats. A peculiarity of FXMD is the presence of calcified nodules on the tongue in addition to tongue hypertrophy. The serum creatine kinase (CK) concentration is usually markedly elevated in dogs and cats with dystrophin deficiency; normal or only mild elevations may be found in manifest female animals. Typical pathologic changes of the dystrophic muscle include myofiber degeneration, regeneration, fibrosis, and calcific

deposits. The diagnosis of a dystrophic myopathy can be confirmed in most instances by the histopathologic evaluation of properly processed muscle biopsy specimens. Absence of dystrophin in immunohistologic analysis provides conclusive diagnosis. Electrophysiological abnormalities have been described, including complex repetitive discharges, positive sharp waves, and fibrillation potentials. These EMG changes tend to increase until 4 months of age. (Bergman et al. 2002)

Specific therapies are not currently available for CXMD or FXMD, and the general prognosis is poor. (Shelton et Engvall 2002)

► **Muscular Dystrophy with Merosin (Laminin α_2) Deficiency**

Congenital muscular dystrophies (CMDs) in human beings are a heterogeneous group of autosomal recessive diseases manifest at birth or during infancy with muscle atrophy, hypotonia, weakness, and contractures. Approximately 50% of human CMD patients have a deficiency of merosin (laminin α_2) expression in muscle. Laminin α_2 is the major component of the basal lamina that surrounds each muscle fiber. Laminin α_2 is one of the extracellular ligands for the dystrophin-associated glycoprotein complex; it links dystrophin to the extracellular matrix and contributes to the stability of the muscle basement membrane. (Shelton & Engvall 2002). Laminin α_2 related CMD is caused by mutations in the LAMA2 gene, encoding the α_2 heavy chain of the laminin 211 isoform ($\alpha_2/\beta_1/\gamma_1$), also known as merosin. In the genetic nomenclature, this CMD subtype is also referred to as MDC1A. Complete absence of laminin α_2 staining on muscle (or skin biopsy) is more common and in general associated

with a more severe non-ambulatory phenotype compared to a partial laminin α_2 deficiency. (Bönnemann et al. 2014) Laminin α_2 is also found in the basement membrane of Schwann cells and in blood vessels within the brain as well as in other tissues. Most of the cases of laminin α_2 -deficient CMD are associated with mutations in the laminin α_2 gene (LAMA2). Peripheral nervous system involvement in primary laminin α_2 deficiency is suggested by reduced motor nerve conduction velocity. (O'Brien et al. 2001). O'Brien et al. (2001) described this pathology in two female cats, the cats have shown an elevated value of creatine kinase (CK). Histopathologically they found dystrophic changes were present in all skeletal muscles examined. Endomysial fibrosis was marked in both cats. In addition, muscles from both cats showed myofiber necrosis, variability of fiber size and perimysial lipid accumulation.

Laminin α_2 -deficient CMD was also described in two Great Dane dogs referred for *quadriceps femoris* contracture. The pathological features described in muscle biopsies are similar to primary merosin deficiency in humans (Trapani F. et al. 2010).

The histologic phenotype of a dystrophic myopathy was determined by evaluation of muscle biopsy specimens, and the exact classification of the muscular dystrophy was confirmed by immunohistochemical analysis. Laminin α_2 is also found in Schwann cell basement membrane and is thought to play a role in ensheathment and myelination of the peripheral nerve. For the dystrophin-deficient muscular dystrophies, no specific therapy is available for laminin α_2 deficiency, and the prognosis is poor. (Shelton et Engvall 2002). And also for the diagnosis in human Sewry et al. (1996) state that human skin expresses the α_2 chain of laminin,

and this tissue can be used to identify patients with congenital muscular dystrophy with a deficiency of the α_2 chain. Muscle biopsy specimens from both cases showed a deficiency of laminin α_2 , similar to that observed in other merosin-deficient cases. The deficiency of the α_2 chain in the skin therefore confirmed the results obtained from the muscle samples.

MD associated with complete or partial loss of dystrophin and dystrophin-associated proteins or laminin α_2 deficiency has been documented in female purebred and mixed-breed dogs. Although muscular dystrophy in dogs has generally been thought of as a disease affecting only young purebred males. (Shelton et al. 2001)

► Hereditary Myopathy of Labrador Retrievers

An inherited myopathy affecting male and female yellow and black Labrador Retrievers less than 6 months of age was first described in 1976. Affected dogs had reduced muscle mass with a poor conformation, a stiff “bunny hopping” gait, and abnormal head and neck posture. Clinical signs were exaggerated with exercise, exposure to cold temperatures, or excitement but did not worsen with age but rather stabilized at about 1 year of age. Evaluation of muscle biopsy specimens revealed a predominance of type 1 fibers with a paucity of type 2 fibers; hence, the description of this disorder as a type 2 fiber deficiency. Further studies documented the inheritance pattern as autosomal recessive in nature.

As a result of the diverse pathologic changes that may be present in muscle biopsy specimens from dogs with a similar clinical presentation, including myopathic and neuropathic abnormalities, the hereditary myopathy of Labrador Retrievers (HMLR) disorder has been variably referred to as muscular dystrophy, myotonia,

polyneuropathy, and hereditary myopathy. Immunohistochemical studies using established monoclonal and polyclonal antibodies against dystrophin, dystrophin associated proteins, and laminins have failed to identify any of the previously described protein deficiencies associated with human or canine muscular dystrophy as the basis for this disorder. The presence of angular atrophied fibers of both muscle fiber types and a type 1 fiber predominance observed in many of the affected dogs is, however, highly suggestive of neuropathy, although pathologic abnormalities of peripheral nerve or spinal cord have not yet been identified. (Shelton et Engvall 2002)

HMLR is not the only muscle disease affecting Labrador Retrievers. The recent identification of dystrophin-deficient muscular dystrophy in a young male Labrador Retriever emphasizes the importance of a correct diagnosis and classification of the muscle disorder, as the mode of inheritance and prognosis differ between HMLR and CXMD. Dogs with HMLR tend to stabilize in clinical severity by 1 year of age and may be acceptable pets, although exercise capacity is limited. This is in contrast to the poor prognosis of CXMD. Similar to the previously described muscular dystrophies, no specific therapies are available for HMLR. Housing in a warm area has been advised, however, because exposure to cold may exacerbate the condition. Moreover, as muscle carnitine concentrations have been low in a few dogs tested with HMLR, supplementation with L-carnitine (50 mg/kg administered orally twice daily) may be of benefit in improving muscle strength. Because a test for carriers is not available, breeders should be advised not to breed parents or siblings of affected dogs. (Shelton & Engvall 2002)

Recently, muscular disrophy resembling human Becker muscular dystrophy was described in a dog (Baroncelli et al., 2014). Furthermore a sarcolemmal specific collagen VI deficient myopathy was reported in a Labrador Retriever (Marioni-Henry et al., 2014) and a mutation in the PTPLA gene was correlated to the Centronuclear myopathy in the same breed. (Maurer et al, 2012) It may be that immunoistochemical molecular studies will allow a better understanding and a precise characterization of several CMD and FMD.

METABOLIC MYOPATHIES

Many endocrinologic disorders, endogenous and iatrogenic, result in muscle and likely peripheral nerve disease. Endocrine myopathies are a relatively common occurrence in geriatric animals and may present with a variety of clinical syndromes ranging from mild weakness or stiffness to complete collapse. (Platt S.R. 2002)

Myopathy secondary to glucocorticoid excess occurs in Cushing's disease and as a result of chronic exogenous corticosteroid therapy (iatrogenic steroid myopathy). (Platt S.R. 2002) Apart from the development of a pendulous abdomen, decreased muscle mass may be noted around the limbs, over the spine or over the temporal region. Muscle weakness is the result of muscle wasting caused by protein catabolism. Occasionally, dogs with hyperadrenocorticism develop myotonia, characterized by persistent active muscle contractions that continue after voluntary or involuntary stimuli. All limbs may be affected but the signs are usually more severe in the hindlimbs. Animals with myotonia walk with a stiff stilted gait. The affected limbs are rigid and rapidly return to extension after being passively flexed. In some cases passive flexion may be difficult or impossible to achieve because of the persistent muscle tone. Spinal reflexes are difficult to elicit because of the rigidity, but pain sensation is normal. (Herrtage M.E. 2004)

A number of mechanisms have been proposed to explain how corticosteroids (exogenous or endogenous) produce muscle weakness and wasting. Glucocorticoids are highly lipid-soluble and easily partition through the cell membrane into the cytosol. Inside the cell, these hormones bind to specific

cytoplasmic receptors which act by modifying transcription. The major actions of glucocorticoids are to increase muscle protein catabolism and inhibit synthesis of myofibrillar proteins. The weakness and muscle atrophy resulting from corticosteroid therapy is not likely a result of motor nerve damage. Motor nerve conduction velocity and histologic studies of nerve were normal in corticosteroid-treated animals. (Platt S.R. 2002)

The clinical manifestations of Cushing's myopathy are similar to that of exogenous steroid myopathy with muscle atrophy and weakness. Unilateral pelvic limb stiffness has been described as a frequent initial sign, with other limbs becoming gradually involved over time. Severe pelvic limb rigidity and clinical myotonia have been found in a subset of dogs with chronic Cushing's disease. Type 2 fiber atrophy is a consistent abnormality in muscle biopsies from dogs affected with Cushing's myopathy. Deposition of perimysial and endomysial fat has been described. (Platt S.R. 2002)

Muscle weakness occurs frequently in association with hypoadrenocorticism (Addison's disease) in cats and dogs. The weakness is usually generalized and may involve the pharyngeal or esophageal musculature. Hypoadrenocorticism classically includes mineralocorticoid and glucocorticoid deficiency. Adrenal insufficiency impairs muscle carbohydrate metabolism, water and electrolyte balance, muscle blood flow, and adrenergic sensitivity, which are all factors that contribute to the weakness associated with Addison's disease. Hyperkalemia develops with depletion of muscle intracellular potassium, decreased membrane sodium potassium–adenosine triphosphatase activity, and diminished β -adrenergic stimulation of the sodium-potassium pump. Correction of the electrolyte

imbalance and glucocorticoid deficiency usually corrects the clinical weakness.
(Platt S.R. 2002)

In human medicine, myopathy (Mor F. et al. 1987), rhabdomyolysis (Su Yin Lau SY and Yong TY, 2012) and fibromyalgia like syndrome (Kaganov Yet al. 2000) secondary to Addison's disease were described.

MISCELLANEOUS

In this chapter a group of disease of uncertain etiology and pathogenesis were described. They share some clinical features with myopathies, but histopathology have failed until now to achieve a definitive understanding of them.

◇ SWIMMING PUPPY SYNDROME

Swimming puppy syndrome is an uncommon developmental disorder of the motor function, still poorly characterized, seen in dogs and less frequently in cats, at an average age of 15 to 21 days after birth. (Cardilli et al. 2013)

It is also known as swimmer syndrome, flat pup syndrome, splay leg (paraparesis), splay weak (tetraparesis), and myofibrillar hypoplasia and is described as a musculoskeletal disorders in puppies. (Nganvongpanit & Yano 2013)

The etiology of swimmer syndrome is uncertain and difficult to prove. Many hypotheses have been suggested, including hereditary factors, environmental causes, unbalanced diet (excessive protein in the queen's diet), maternal metabolic disorders, musculoskeletal development problems, obesity, and neurological disorders. (Cardilli et al. 2013)

The condition is mostly seen in brachycephalic dog breeds, and an association with *pectus excavatum* has been previously reported. The animals try to ambulate with swimmer like movements, and when positioned on smooth surfaces, the clinical signs are more pronounced. (Verhoeven et al. 2006; Cardilli et al. 2013)

In the initial weeks of life, newborn puppies seem normal: they gain weight quickly, suck well, and appear to be completely healthy. Signs begin to appear

when the puppy learns to walk (2nd-3rd week), with spread out legs like a swimmer. In some cases, there are additional complications, because such puppies tend to lie on their bellies most of the time.

The center of gravity is shifted to the chest, while the soft ribs cannot maintain their correct shape; thus the chest, under the pressure of body weight, splays on both sides, and the thorax becomes flat (funnel chest). Sterna concave, dorsoventral flattening of the chest, or *pectus excavatum* will present when forelimbs are affected. In cases of *pectus excavatum*, puppies show respiratory insufficiency, with dyspnoea, mouth continuously open, bluish mucous membranes, and medial patellar luxation and malformation of articulations of the long bones (*genu recurvatum*). (Nganvongpanit & Yano 2013;Cardilli et al. 2013; Verhoeven et al. 2006)

The results of a study showed that complete blood counts and the levels of most blood chemicals were not useful for diagnosis of swimming puppy syndrome. Although serum CK was elevated in animals with swimming puppy syndrome compared with control puppies and with normal levels, it cannot be used for diagnosis of this disease due to silent elevation. However, CK could be used as a tool for prognosis of the disease and to evaluate the efficacy of treatment. (Nganvongpanit K. 2012)

A definitive diagnosis can be made based on history, clinical signs and radiographic examination. The differential diagnosis of this disease includes encephalomeningitis, canine distemper, toxoplasmosis, neosporosis, myopathies, and spina bifida. The treatment success rate is dependent on the time of diagnosis and treatment. Usually puppies with this disease recover well after early diagnosis

and treatment. Swimmer syndrome treatment should include dietary modifications, relocation to rough surface for motor stimulation, anatomical immobilization of the affected limbs, physical therapy, thermo and hydrotherapy, and massage for muscle strengthening. (Nganvongpanit & Yano 2013; Cardilli et al. 2013; Verhoeven et al. 2006)

◇ CARPAL LAXITY SYNDROME

Congenital deformities are multifactorial (teratogenic agents, intra-uterine mal-positioning, diseases of the mother during pregnancy etc). Acquired deformities can occur due to trauma, infectious polyarthritis and nutrition. Vaughan (1992) has suggested that Doberman Pinschers may be predisposed to carpal flexural deformity, and that this lesion may be hereditary. Altunatmaz & Ozsoy due to the cases in their study belonging to different breeds, and lack a dominant breed, do not agree with the idea postulated by Vaughan.

The carpal laxity syndrome is defined as carpal hyperflexion or hyperextension of the carpal joints. Hyperflexion and hyperextension occur separately or simultaneously on both legs or on only one leg. Poor muscle tone or deficiencies between the extensor and flexor muscles, excessive exercise, ligament deficiencies due to an excessive weight gain before adequate bone development and unbalanced growth may play a role in aetiology of the carpal laxity syndrome, but no alteration in mineral metabolism has been reported. This syndrome in puppies is frequently seen in rapidly growing medium, such as large and giant breeds. Sex was not identified as a risk factor. Carpal hyperflexion or hyperextension was not associated with pain in slight or severe forms. The intense

bone formation suggested by the increase of the plasma ALP activity would be associated with an increased mineral fixation, leading to a normalization of the plasma Ca and P concentrations. It would be also possible that a vitamin D deficiency would promote disequilibrium between bone formation and resorption. Vitamin D metabolites regulate the calcium metabolism and therefore skeletal development in dogs. These metabolites aid in the absorption of calcium and phosphorus from the gut, increase bone cell activity, and influence endochondral ossification and calcium excretion. (Atalan et al. 2009)

Hyperflexion carpal syndrome is caused by the contracture of the flexor carpi ulnaris, The suspected diagnosis, based on the basis of signalment, medical history, general physical examination and orthopedic examination, the diagnosis is confirmed with radiographic examination. The disease, often self-limiting, has a favorable prognosis and a course generally short. The therapeutic approach can be expected, according to the severity and the clinical course, conservative or surgical measures. (Petazzoni & Mortellaro 2000)

In foal, congenital flexural deformities have been attributed to a fetal malposition during intrauterine life. Also in this species other factors invoked are represented by skeletal abnormalities of the period accretion, genetic mutations, ingesting teratogenic substances as well as infectious diseases occurred during pregnancy. (Stashak T.S. 1987)

Vaughan suggests the existence of a development asynchronous between skeletal tissue and tendon-muscular with greater growth of the first segment, due to a relative shortening tendon-muscular and consequently due to hyperflexion and hyperadduction carpal.

Dogs affected by SIC are generally very young (6-16 weeks of life) and they are of medium, large or giant size, the disease is reported in numerous breeds. The pathology is usually bilateral, although the two forelimbs may be involved in different times and with varying severity; rarely can be also affected a single limb. The anamnesis does not report injuries. (Petazzoni & Mortellaro 2000)

Bandage application was considered to be helpful to improve osteogenesis by diminishing constraints on bones and provided the shortening of the laxated flexor and extensor tendons. Although this syndrome may spontaneously be attenuated, some treatment methods such as balanced diet, moderate and appropriate exercises, the Robert Jones bandaging, splint, tenotomy and arthrodesis have been reported. (Atalan et al. 2009)

◇ QUADRICEPS CONTRACTURES

Quadriceps Contracture (QC) is a disease which is seen as a congenital deformity or as a complication of fractures of the femur in juvenile dogs. (Montgomery & Fitch 2003; Ulsan et al. 2011) This disease is also known as post-traumatic stifle joint rigidity, quadriceps tie-down syndrome, stifle joint hyperextension, hindlimb rigidity, quadriceps ischemic contracture or Sudek atrophy. QC has been reported as a complication from toxoplasmosis and neospora infection (Ulsan et al. 2011; Taylor J. & Tangner C. H. 2007) Factors that promote the likelihood of quadriceps contracture are age less than 6 months and splinting of the leg (especially in extension), although quadriceps contracture can develop without these factors. Contracture occurs over a period of weeks and, when complete, results in the stifle and hock locked in full extension. Most dogs with quadriceps

contracture are not able to place the foot on the ground. Marked atrophy of the quadriceps is followed ultimately by general atrophy of the other muscle and the bone, typical caused by disuse. Treatment is effective only when implemented early and should be directed at early motion of the muscle and decreasing scar tissue during muscle healing. Salvage techniques include release of the quadriceps muscle group from proximal femur and pelvis, arthrodesis of the stifle and hock, and amputation. Arthrodesis and amputation are the only viable options when the condition is severe. (Montgomery & Fitch 2003)

In case of infection by *Neospora* or *Toxoplasma* the treatment consist in trimethoprim-sulfadiazine (TMS), and pyrimethamine. TMS at a dosage of 15 to 20 mg/kg administered orally twice daily in combination with pyrimethamine at a dosage of 1 mg/kg/d is one treatment option. Alternatively, clindamycin at a dosage of 15 to 20 mg/kg administered orally twice daily, with or without pyrimethamine, is also recommended. Another possible combination therapy would be TMS and clindamycin at the dosages stated previously. Treatment should continue for 4 to 6 weeks.

EXPERIMENTAL STUDY

INTRODUCTION

Myopathies can be classified as inflammatory or degenerative in nature. Inflammatory myopathies are the result of infiltration of inflammatory cells into striated muscle, with or without an association with an underlying cause. Inflammatory myopathies can be classified into two broad categories as seen in the list on the next page: idiopathic inflammatory myopathy (IIM) and secondary inflammatory myopathies associated with other diseases. Clinical signs for both types of inflammatory myopathy are related to the degree of inflammation, number and location of affected muscles, and presence of systemic disease. The ability to arrive at a definitive diagnosis for a successful therapeutic approach is dependent on early recognition of the clinical signs, appropriate diagnostic testing, and interpretation, followed by specific therapy either to immunosuppress the patient for IIM or to treat with anti-infective agent for infectious-induced myopathies. (Podell 2002)

Inherited muscle diseases in dogs and cats are relatively uncommon and may be difficult to diagnose. It is important to obtain a correct diagnosis in these types of diseases because most occur in purebreds and knowledge of inheritance patterns is of utmost importance to animal breeders. A correct diagnosis is also important for animal owners, as the prognosis differs for the various muscle diseases. (Shelton & Engavall 2002)

Standardized early examinations, comprehensive of serum creatine-kinase (CK) and metabolic investigations, neurophysiology (electromyography and nerve conduction velocity studies), neuroimaging (cranial ultrasonography and magnetic resonance imaging) and muscle or nerve biopsy of affected children is mandatory

to a better understanding of the underscored disorder and to begin the most appropriate therapy (Vasta et al., 2005).

Aims of the study were to describe a case series of myopathies, in order to suggest a diagnostic algorithm and to verify the clinical usefulness of the muscular biopsy in the diagnostic *iter*.

MATERIALS AND METHODS

This is a retrospective study in which we evaluate all the cases of myopathies referred to the Department of Veterinary Medicine and Animal Productions of the University of Napoli Federico II from 2004 to 2014. We collected clinical cards listed on our computerized archiving system considering the species, breed, age, size and weight. As regards the history we focused especially on the timing of onset and then evaluating whether present from birth or later developed. At the same time we tried to assess whether clinical signs consistent with myopathy were the reason for the visit by the owner. So we performed the clinical examination and evaluation of the main symptoms that the animal showed. Most of the time the animals showed weakness, tremors, lameness, pain on muscles palpation. The diagnostic protocol proposed to the owners consisted of: complete blood count, serum biochemistry panel, evaluation of the IGG against *Neospora* and *Toxocara*, urinalysis, radiographs, electrophysiologic evaluation and muscle / nerve biopsies.

RESULTS

During the examined period, a total of 63 subjects were identified as affected from a neuromuscular disorder, two of which were cats (1,3% whereas the remaining were dogs (98,7%) (fig. 1). Considering a probability test with an equal frequency (50%) of neuromuscular disorders between the two species, the distribution resulted significantly different ($P < 0.0001$).

Figure 4

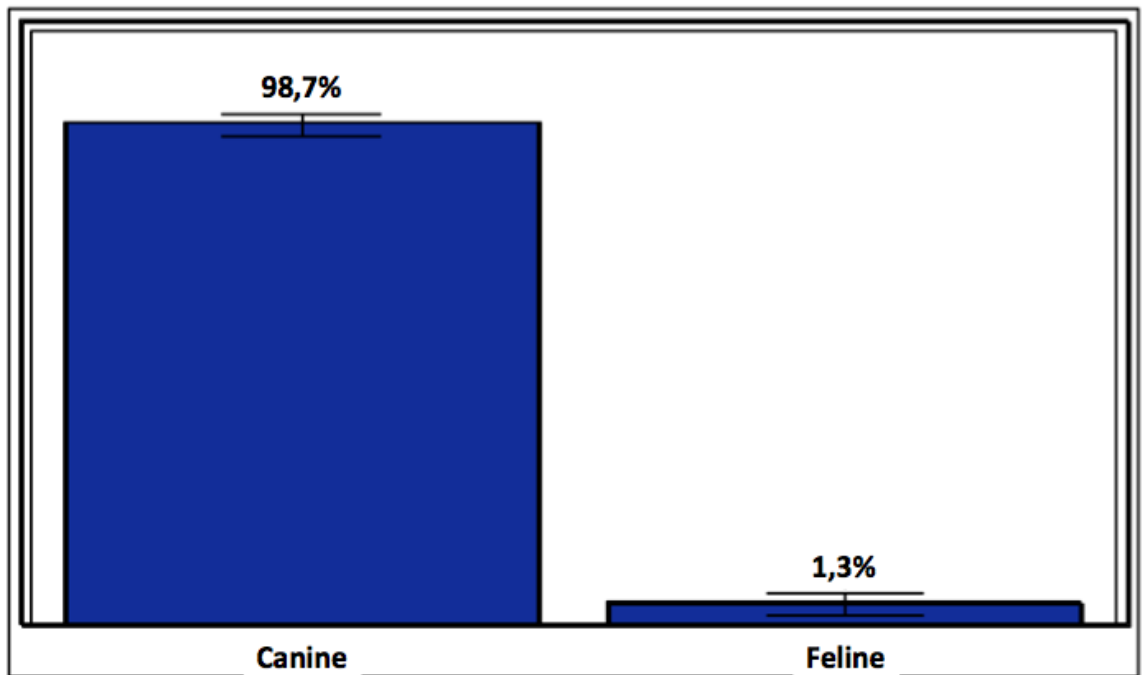


Figure 4: Frequency histogram of the sample studied considering the species. 1

The canine sample belonged to 25 different pure-breeds even if the most abundant portion belonged to mixed breed (28%). The two cats were European Shorthair.

Male patients represented almost half of the sample (57%); when testing the probability of having a neuromuscular disorder independently of the sex, the difference was statistically significant ($P < 0.0001$) (fig. 5).

The mean \pm SEM age of onset was 41 \pm 5 months (median 18 months; range 1-180 months), even if the most of the neuromuscular disorders were recorded in the first year of life (50%) (fig.6).

The most of the patients were large sized breeds (47%), and in fact the prevalence in such class resulted significantly more probable ($P=0.002$) (fig. 7).

The most frequent cause for the owners to take their pet to be visited was weakness (33%), followed by lameness and deformity of one or more limbs (27%, both); trismus (6%), shivering (3%) or gastrointestinal/general symptoms (3%), i.e. vomiting, diarrhea and anorexia, were the least frequent reported signs (fig. 8).

Clinical examinations revealed various signs, summarized in figure 9.

EMG was performed in a small number of cases (13). No abnormalities were shown in the tested cases of polymyositis and MMM, except for a decrease of the insertional activity in presence of severe fibrosis. In the myopathy secondary to Addison disease no abnormalities were detected as well. In the case of neurogenic myopathy associated to mitochondrial activity deficit, EMG showed an increased insertional activity at the *vastus lateralis m.* and at the *tibialis cranialis*, fibrillation potentials (50 to 200 μ V), sharp positive waves (100 to 200 μ V), bizarre high-frequency discharges and at the right *gastrocnemius m.*. Nerve conduction velocity (NCV) was reduced (44,7 m/s). Decreases of NCV and occasional bizarre high-frequency discharges were the main features of neuromuscular pathologies and of the polyradiculoneuritis.

Histology resulted in a definitive diagnosis in 56 (71%) of cases. The most frequent pathology was neurogenic myopathy (30%), followed by inflammatory myopathy (14%), muscular dystrophy and aspecific myopathy (13%, both) (fig. 10). In a single case (laxity) the sampling was incorrect and only fascial tissue was collected . On the basis of clinical and histopathological data the following class of disease were diagnosed: neurogenic myopathy (14), focal myositis (2 cases), polymyositis (10), MMM (5), metabolic myopathy (3), aspecific myopathy (2), traumatic myopathy (4), myasthenia (4), muscle dystrophy (4), mitochondrial myopathy (1), poliradiculoneuritis (1), swimming puppy syndrome (4), SIC (4), primary quadriceps contracture (6).

Focal myositis involved the proximal muscles of the left hind limb in one case and the *quadriceps femoris m.* in the other case. In the first case the pathology was associated to a sierological diagnosis of ehrlichiosis and the inflammation resolved after specific therapy (doxycycline 20 mg/kg die for 21 days). In the latter case an underlying cause was not identified and the symptoms partially regressed after non steroidal anti-inflammatory treatments and rest. MMM, in one case was incompletely recognized, due to the prevalence of fibrotic tissue degeneration.

Etiology of the polymyositis and of the radiculoneuritis remained uncertain in most cases. Four cases of polymyositis and 2 of MMM were associated to leishmania infection.

Traumatic myopathies involved *semitendinosus m.* (1 case), *infraspinatus m.* (1), *pectoral muscles* (1 case), *adductor mm of the thigh* (1 case) .

Metabolic myopathies were associated to Cushing syndrome in 3 case and to Addison disease in 1 case.

MD was identified as alfa 2-laminin deficiency in two Great Danes, whereas dystrophy was not classified in two biopsies from an Akita Inu litter. In this case, muscle lesions were identified in the clinically healthy queen, and in one of four 2 months old puppies. All the puppies showed severe weakness and amyotrophy. Immunohistochemistry failed to identify the deficiency of the tested proteins.

Mitochondrial myopathy was diagnosed as main histopathological lesion in a 9 years old cross breed dog, showing stiffness and exercise intolerance. Mitochondrial activity deficiency was associated to neurogen myopathy in a 5 years old male fox terrier. In this case stiffness, exercise intolerance and shivering were the main clinical features and corticosteroid treatments was followed by the regression of symptoms, confirmed at a 6 months clinical follow-up.

Aspecific myopathic changes were demonstrated in two cases of swimming puppy syndrome and in 2 case of SIC.

Neospora canis was sierologically diagnosed in a case of *quadriceps femoris* contracture. In the other four cases sierological tests for *Neospora canis* and *Toxoplasma gondii* were negative and aspecific alterations were observed at the histological stains.

Neither the histological nor the clinical diagnosis correlated with age of onset. Nonetheless, considering histological diagnosis (fig. 11), subjects affected by muscular dystrophy (mean 11 months old) were significantly younger ($P=0.04$) than patients with neurogenic myopathy (mean 84 months old). On the other hand, considering the clinical diagnosis (fig. 12), subjects affected by swimming puppy

syndrome (mean 1 month old), by carpal hyperflexion and hindlimb hyperextension syndrome (mean 9 month old, both) were significantly younger than both muscular atrophies (mean 72 months old) ($P=0.04$, $P=0.04$ and $P=0.005$, respectively) and pure post-traumatic myopathies (mean 158 months old) ($P=0.01$, $P=0.03$ and 0.009 , respectively).

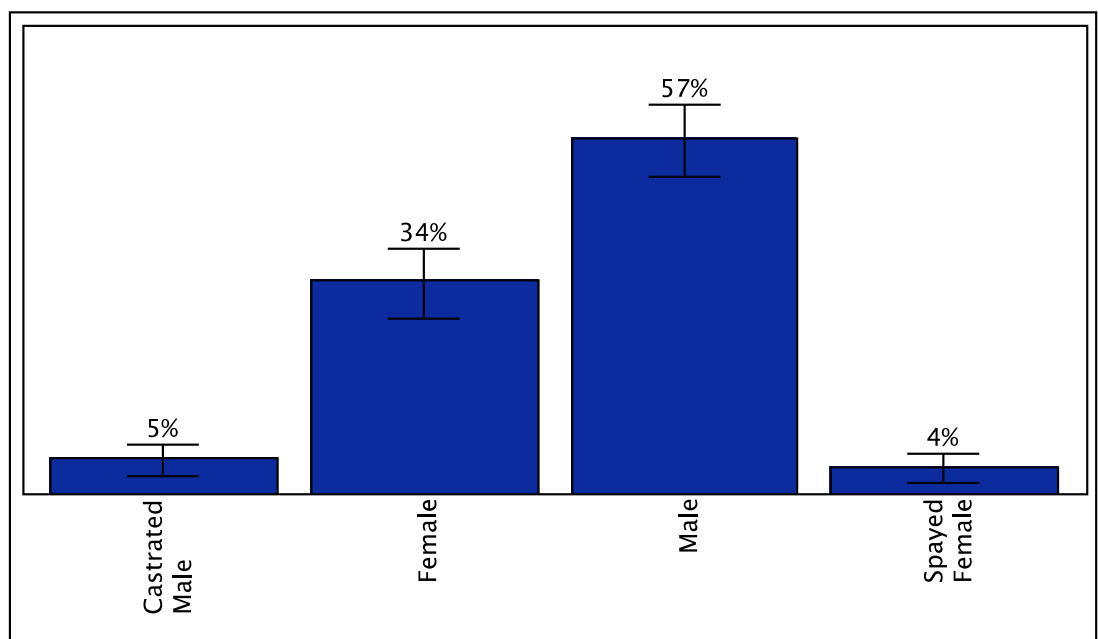


Figure 5: Frequency histogram of the sample studied considering the sex.

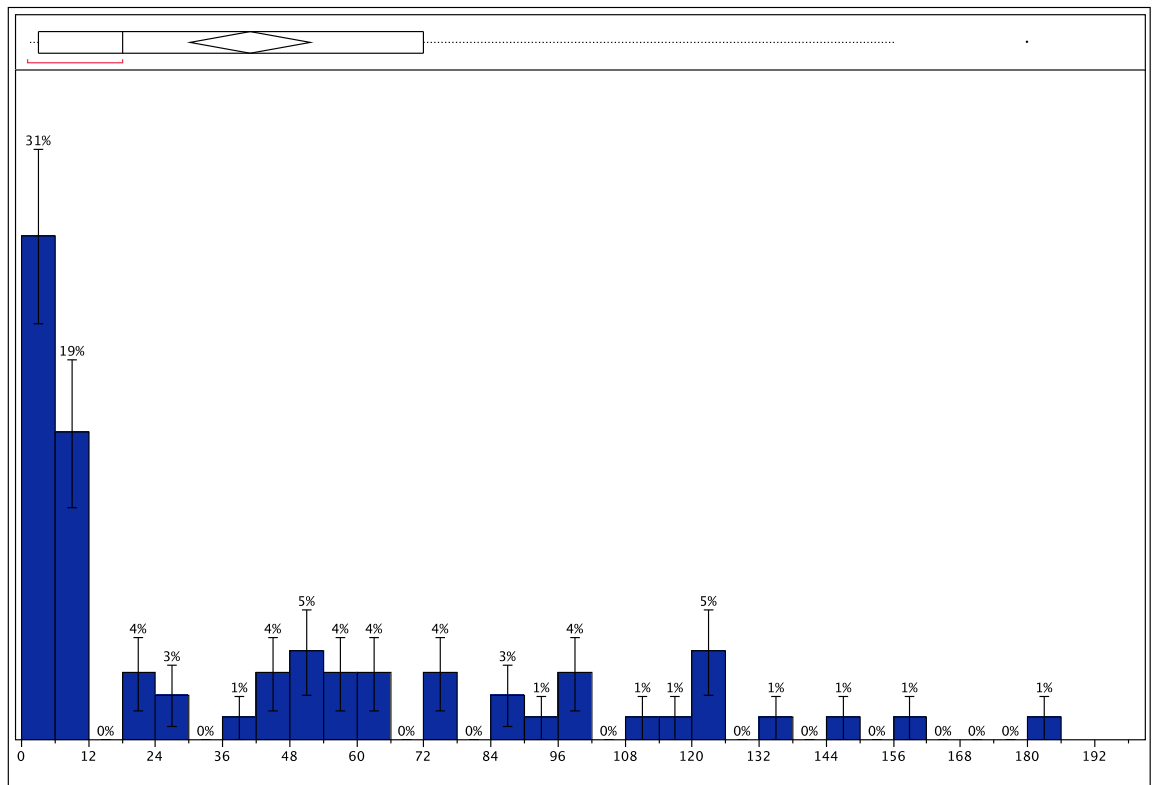


Figure 6: Frequency histogram of the sample studied considering the age of onset.

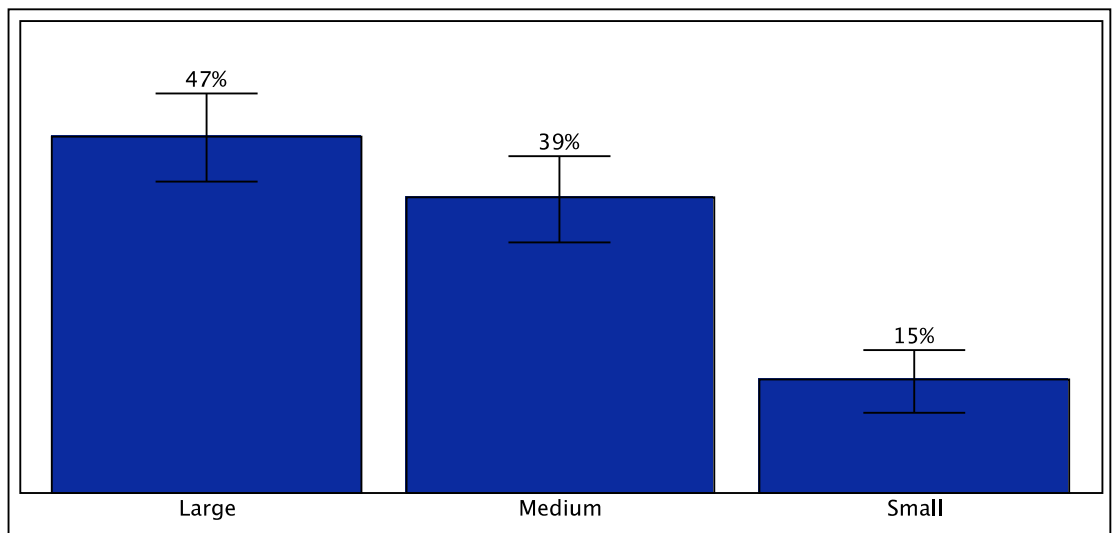


Figure 7: Frequency histogram of the sample studied considering the size.

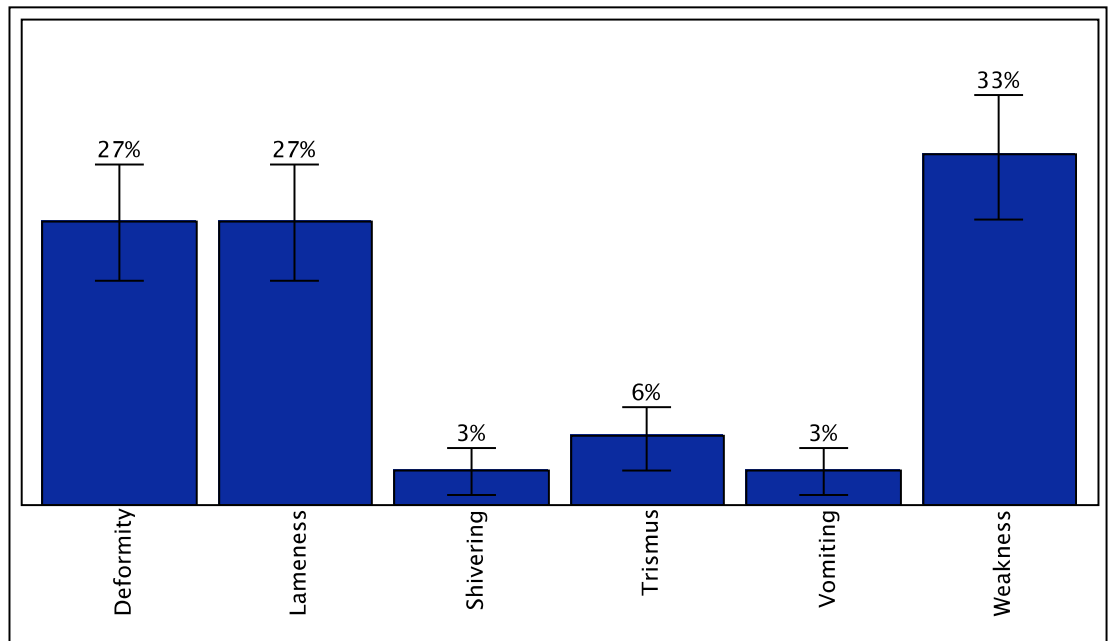


Figure 8: Frequency histogram of the symptoms identified by owners.

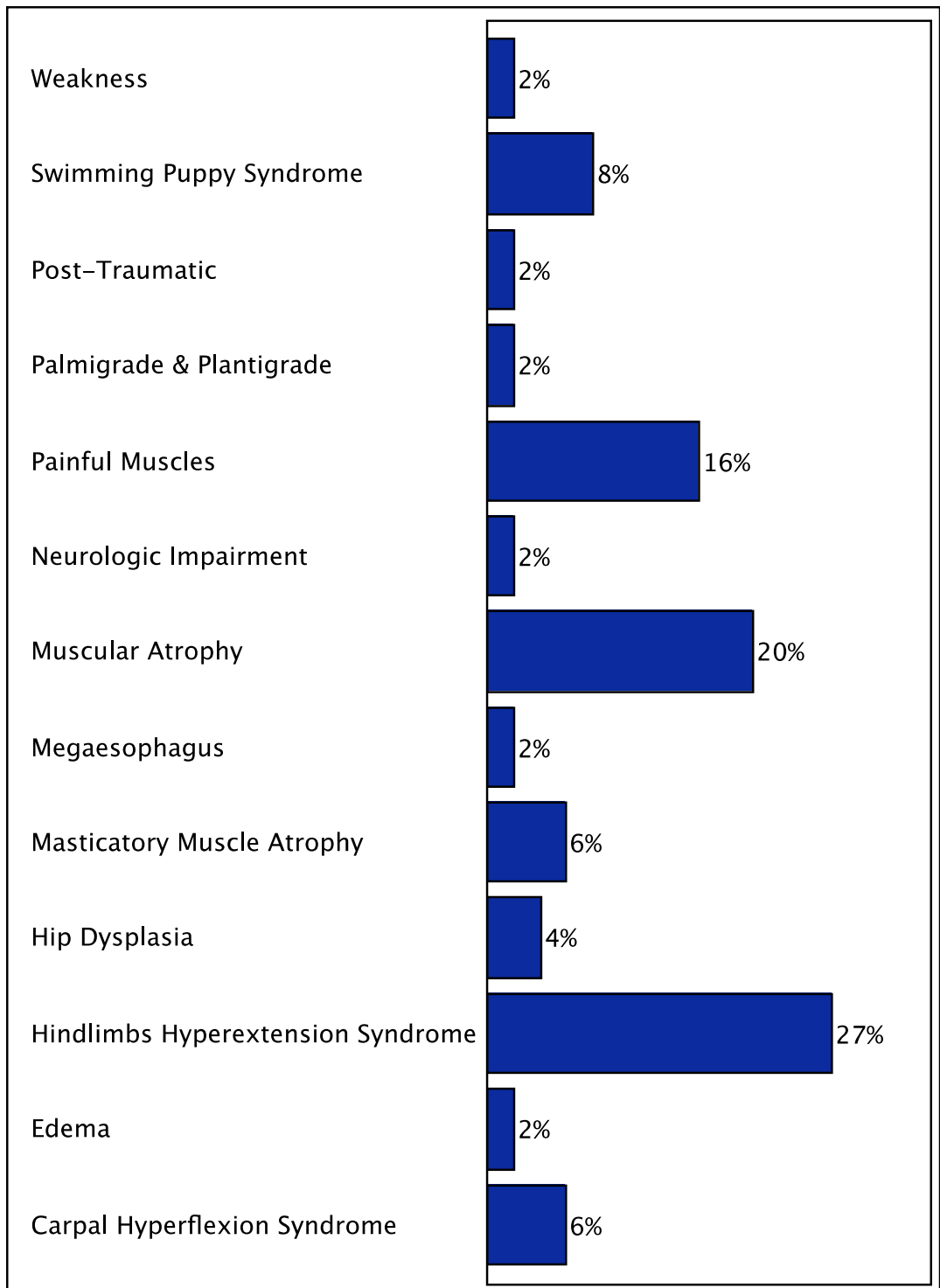


Figure 9: Frequency histogram of the signs identified by at the clinical examination.

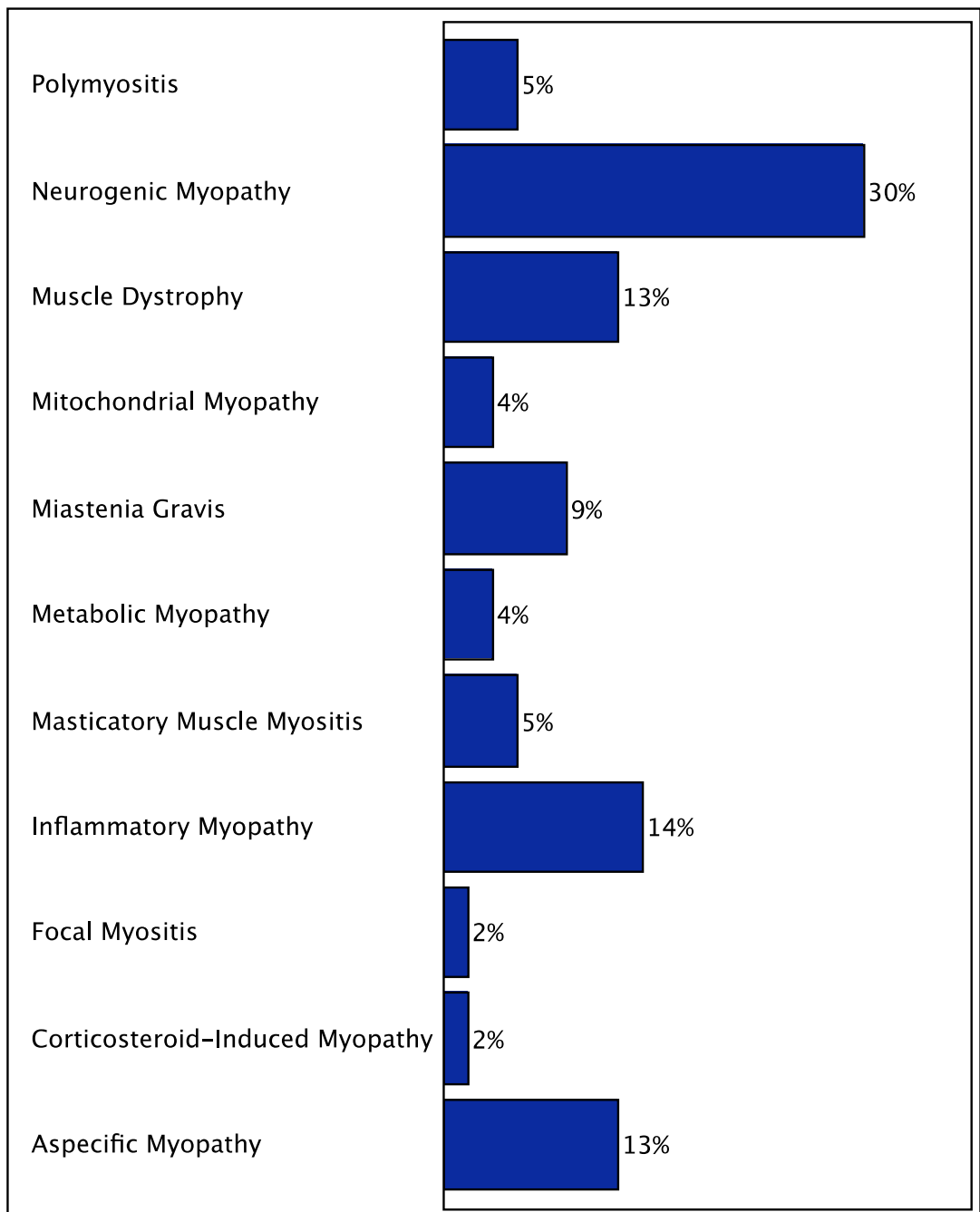


Figure 10: Frequency histogram of the histopathological diagnosis performed on muscle biopsies.

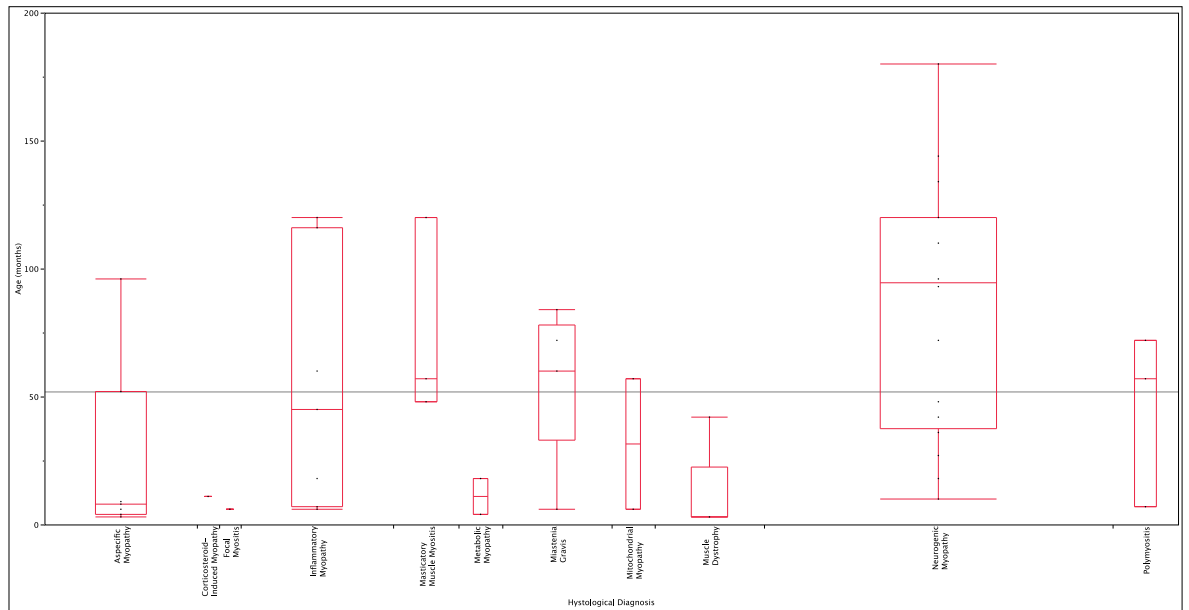


Figure 11: Box plots of age (months) considering the histopathological diagnosis.

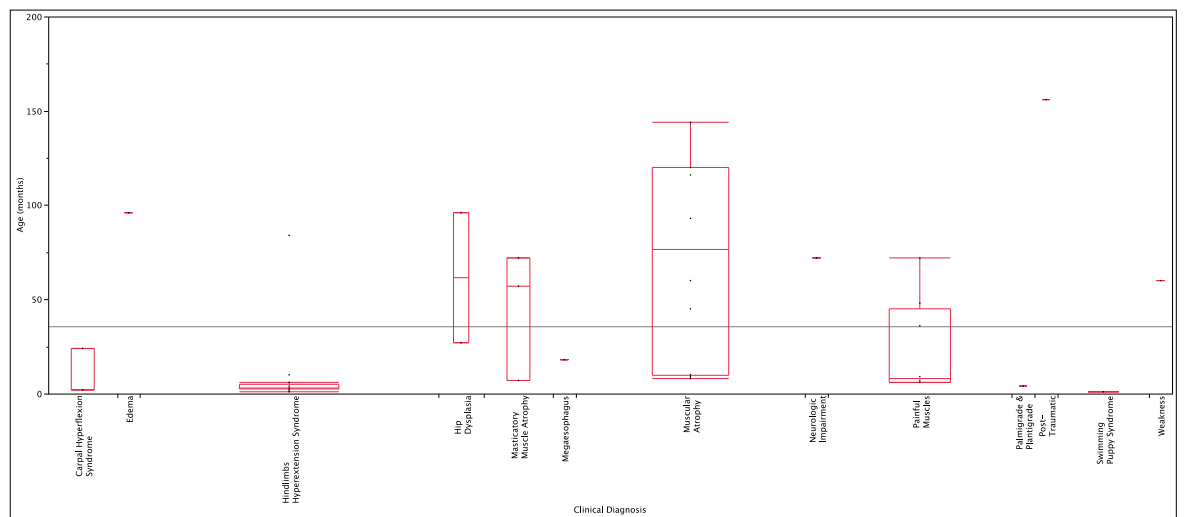


Figure 12: Box plots of age (months) considering the clinical diagnosis.

When considering the correlations between histological and anamnestic data, deformities resulted significantly associated with muscle dystrophy, shivering with mitochondrial myopathies and myasthenia gravis, trismus with masticatory muscles myositis (even if it was recorded also in inflammatory myopathy and in polymyositis) ($P<0.0001$) (fig. 13).

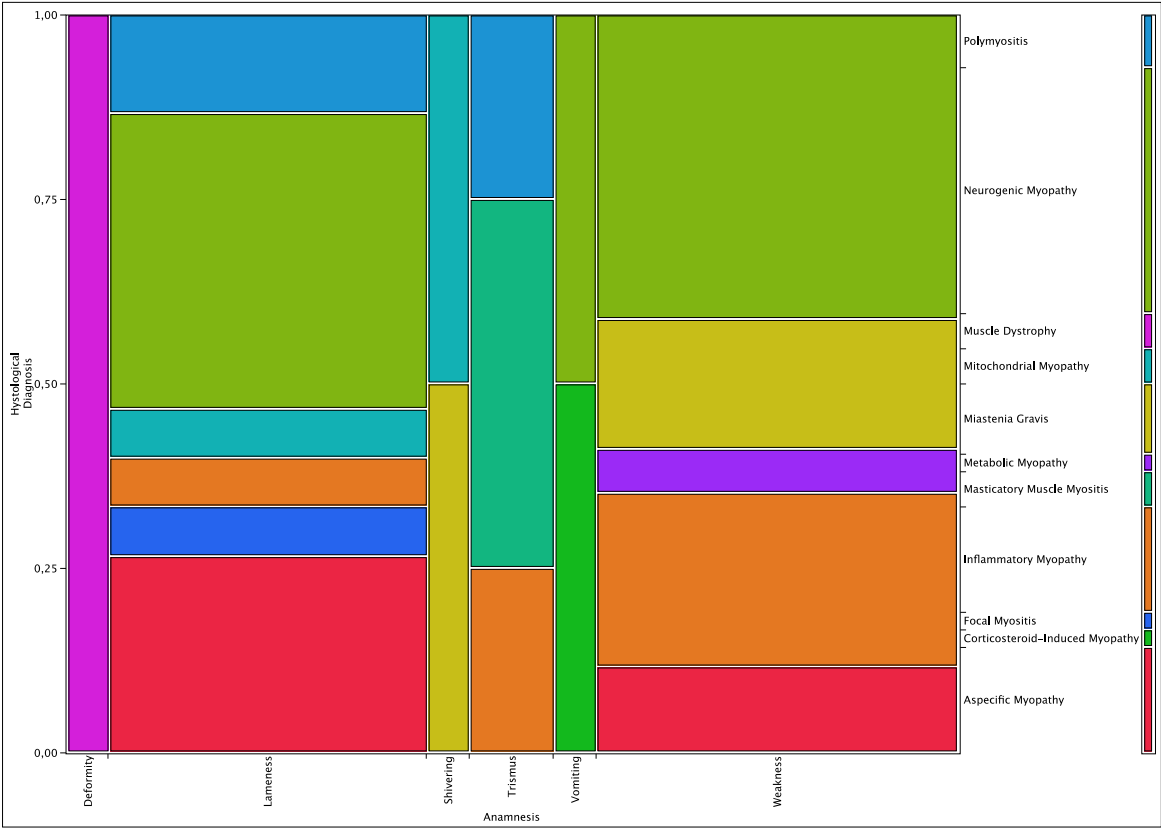


Figure 13: contingency table evaluating correlations between anamnestic (x axis) and histopathological (y axis) data.

No specific correlations were found between histological and clinical diagnosis
($P=0.68$) (fig. 14).

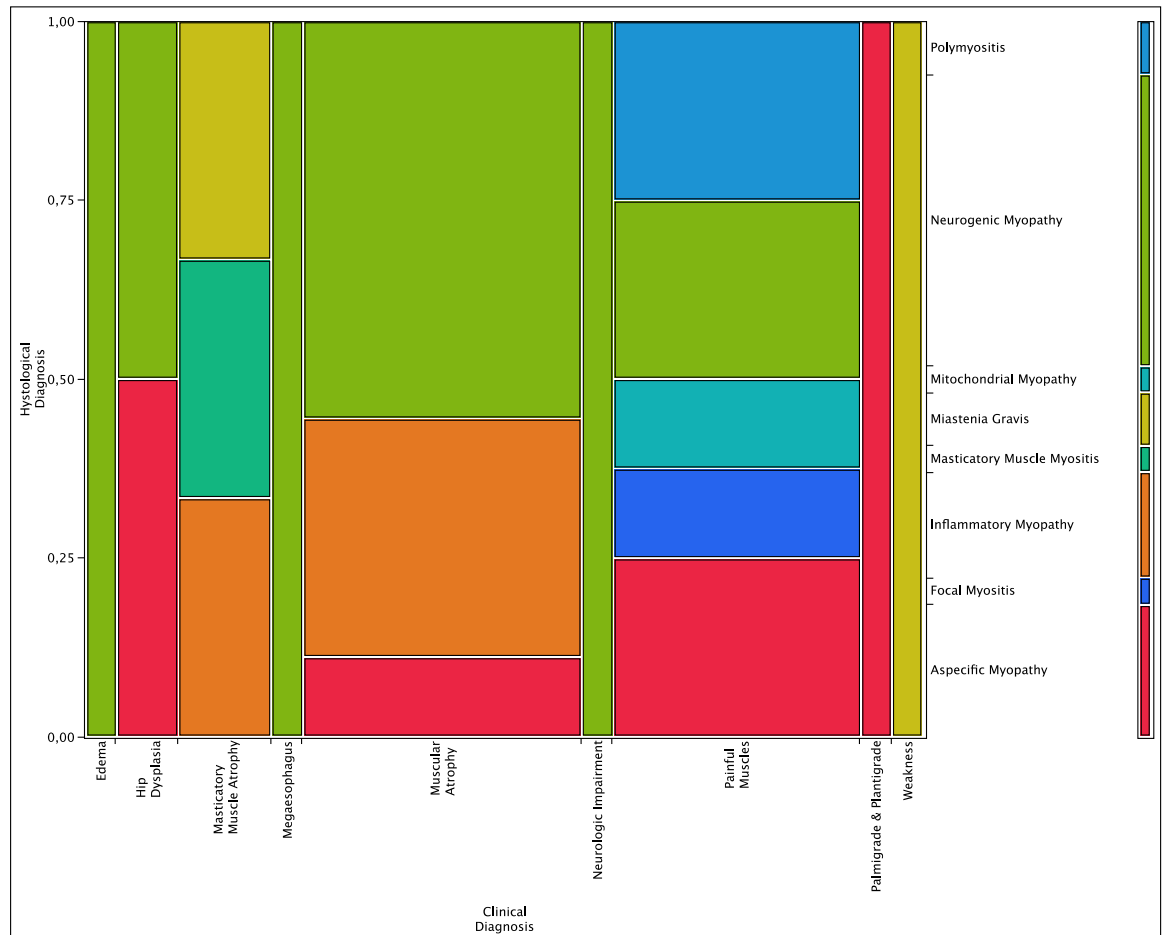


Figure 14: contingency table evaluating correlations between anamnestic (x axis) and clinical (y axis) data.

Muscle dystrophy resulted over-represented in Akita Inu and Great Dane, and in both cases it was a familial form; myasthenia gravis was more frequent in Bull Terrier and Pointer; Focal myositis in Dalmatian; Masticatory Muscles myositis in Shar Pei ($P=0.01$) (fig. 15).

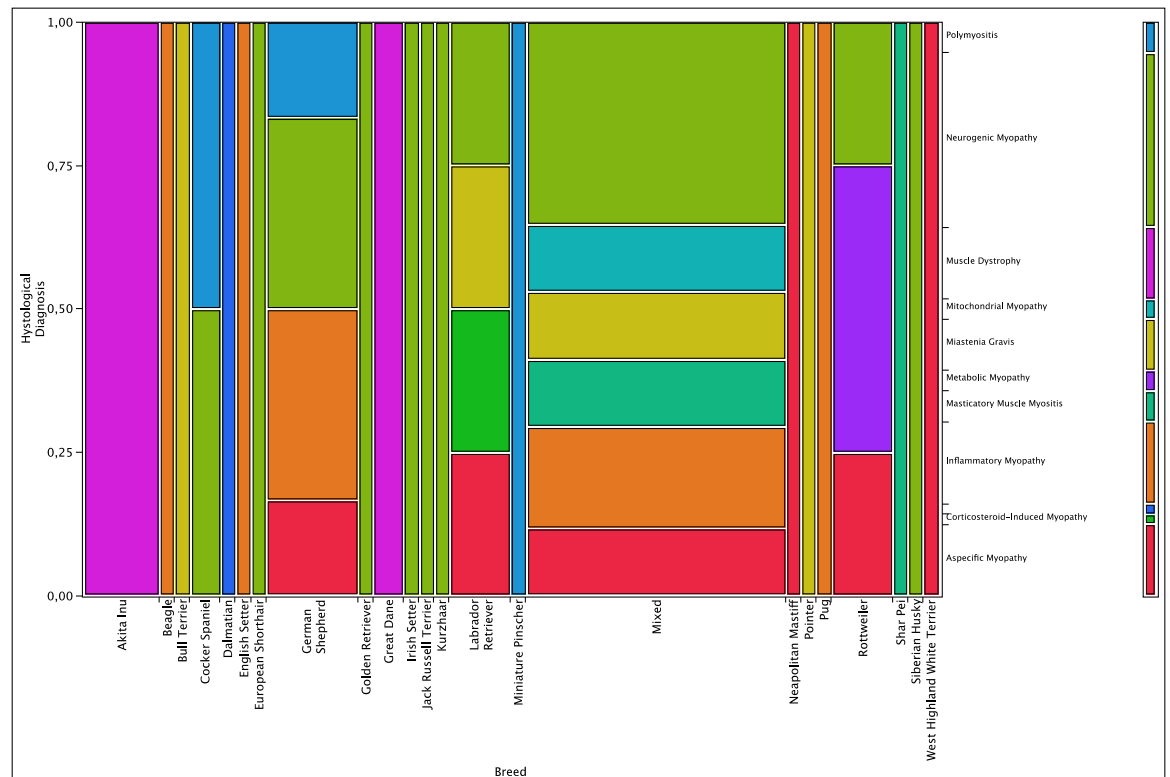


Figure 15: contingency table evaluating correlations between breed (x axis) and histological diagnosis (y axis).

Considering the clinical diagnosis, Carpal Hyperflexion Syndrome was over-represented in American Pitt Bull Terrier ($P=0.003$) (fig. 16).

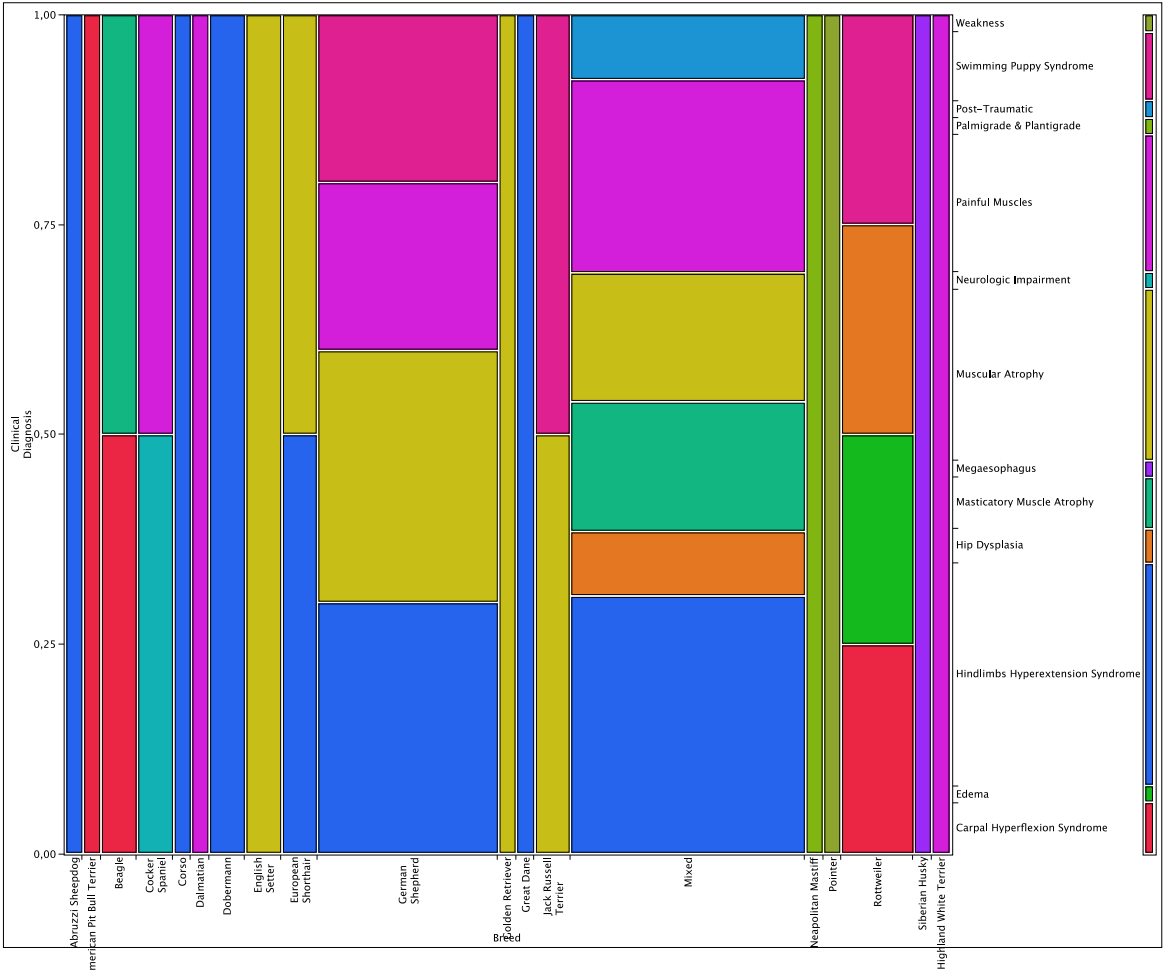


Figure 16: contingency table evaluating correlations between breed (x axis) and clinical diagnosis (y axis).

DISCUSSION

Neuromuscular disorders are emerging pathologies in small animal clinic. A case series of 63 small animals affected by neuromuscular disorders is reported. The prevalence of dogs compared to the cat correspond to the literature data. Even if no study compares the prevalence of canine and feline neuromuscular disorders, literature about congenital and acquired diseases is wider and richer in dogs than in cats. However, it is known that myopathies and neuromuscular disease present different etiopathogenetic and clinical features in the two species (Braund, 2006). Furthermore, disorders affecting motor neurons and nerve roots, peripheral nerves, neuromuscular junctions, and muscles can have a similar clinical appearance and in the cat, as well only in the dog, a complete diagnostic iter allow to obtain a precise diagnosis. (Ginman A. A. et al. 2009) Thus, conclusions of this study are referred exclusively to the dog and data cannot be extrapolated for other species.

Congenital neuromuscular disorders are often described in specific breeds and a genetic basis is postulated for many of them (McGreevy J.W. 2015; Shelton G.D. 2005; Ambrósio C.E. 2009; Broeckx B. J. G. 2013; Switonski M. 2014; Kornegay J.N. 2014). In this series, about a third of the cases are cross breed dogs, according to the population from which the group was extrapolated. In literature Evans et al. (2004) in a study of 200 cases of inflammatory myopathies Boxer and Newfoundland were overrepresented. MD is historically related to Labrador Retriever (Bley T. 2002; Bergman R.L. 2002; Baroncelli 2014; Watson A.D. 1988; Kommonen 1990;), but nowadays it is reported in different and always new breeds. Other examples of congenital myopathies breeds related include:

mitochondrial myopathy, reported in Clumber and Sussex spaniel (Herrtage E. 1979; Houlton 1980), German Shepherd, Old English Sheepdog, Jack Russell Terrier and Labrador Retriever (Paciello O. 2003; Tauro A. 2008; Olby 1997); Congenital Myotonia, reported in Chow Chow, Staffordshire terrier, Great Danes and Miniature Schnauzer; Dermatomyositis, reported in Shetland Sheepdog, Beauceron Shepherd, Pembroke Welsh Corgi, Australian Cattle dog, Lakeland terrier, Chow Chow, German Shepherd and Kuvasz. In some cases, specific myopathies are reported only in one breed, as for Bouvier des Flandres Myopathy (Braund 2006; Braund 1990; Peeters M.E. 1991). In these cases a genetic etiology or a breed predisposition is postulated. Specific mutations have been identified in CNM and XLMTM in Labrador Retrievers (Shelton 2010), whereas X-linked myotubular myopathy in Rottweiler dogs is caused by a missense mutation in Exon 11 of the MTM1 gene (Shelton et al. 2015) and Centronuclear myopathy in Labrador retrievers was correlated to a mutation in the PTPLA gene (Maurer et al. 2012). Unfortunately, genetic molecular tests are not easily available in practical settings and in this case series we cannot identify a genetic etiology in any case.

However, in some cases similar clinical, electrophysiological and histopathologic features are shared with acquired diseases, described in adult mix-breed dogs. For example, myotonia is described as an acquired disease, secondary to endocrinological disorders, such as Cushing disease and hypothyroidism. In this case no breed predisposition is reported (Braund K.G. 1980; Greene C.E. 1979; Rossmeisl J.H. 2009; Braund K.G. 1981).

Age of onset of our cases, is related to the disease prevalence as well. Swimming puppy syndrome, SIC, Quadriceps contracture and MD are obviously reported in puppies or in very young dogs. The clinical onset in a young dog of disease characterized by weakness, exercise intolerance and/or contracture should be considered suggestive of a congenital neuromuscular disorders. As above indicated, a complete diagnostic iter should be triggered in these cases, whenever possible, on the basis of the owner consent and of the availability of diagnostic means.

Blood chemistry showed a wide range of results, correlated to the variability of the underlying diseases. Sierological muscle markers were significantly raised only in few cases. CK, AST and LDH are considered sierological markers of muscle damage. The plasma half-life of CK is shorter than half-life of AST and serum AST increases are delayed by 36-48 hours after a muscle damage, when CK levels tend to normalize (Shelton 2010). CK activity could be related to the nature of the diseases, but to their chronicity as well, and to the absence in many cases of acute fibers necrosis. Thus, due to the heterogeneity of this case series, a wide range of activity of the muscle markers was predictable. However, hematochemical analysis was useful in excluding or confirming metabolic and secondary myopathies and in monitoring therapy results.

EMG and histopathology were proof to reveal a peripheral nervous system impairment probably due to a mitochondrial dysfunction in the 5 years old fox terrier. Electrophysiology was a useful mean to characterize or to exclude muscle and neuromuscular disorders, whenever was available. It was shown that EMG can give information about the extent and etiology of neuromuscular disease, but

that it has limited value in inferring clinical symptoms and neuropathic deficit, distinguishing between neuropathy and myopathy, inferring involvement of small-diameter fibers, inferring underlying biochemical or other pathophysiologic derangement, inferring the presence and type of pathologic alterations in single fibers and Schwann cells, if not related to anamnestic, clinical and histopathological data. Despite these limitations, EMG is clinically useful in differentiating between denervation atrophy and disuse muscle atrophy. (van Nes 1986; Cuddon 2002)

Histopathology was almost always determining in the definitive diagnosis. The sample has to be correctly collected and sent to a specifically equipped laboratory. Biopsies of muscle, and in some cases peripheral nerve, should be collected early in the course of diagnostic evaluation of an animal with a neuromuscular disease, rather than waiting until extensive muscle damage, fiber loss, and fibrosis have occurred, when the chances for a successful treatment are diminished. Delay in diagnosis and initiation of appropriate therapy may result in irreversible fibrosis and limb contractures and fibrosis prevents in achieving useful diagnostic information about the primary disease. (Shelton 2010)

Even if Labrador retrievers are considered an important animal model for human MD, complete antibodies panel for the immunohistochemical diagnosis of canine dystrophies are expensive, not easily available and not always validated for this species. (Shelton, 2010) For these reasons, in our case series, some cases were classified as MD, on the basis of the histopathology, but not further defined. On the contrary, it was possible to obtain a definitive diagnosis for the two Great Danes puppies, affected by $\alpha 2$ -laminin deficiency. This is the first documented

case of this disease in the dog (Trapani et al., 2010). It is interesting the correspondence between the severity of the clinical presentation and the expression of the antigen-antibody reaction at the immunohistochemistry, that was almost completely absent in the male dog, affected by a bilateral contracture of the *quadriceps femoris m.*, associated to hydrocephalus and a severe blood-coagulation deficiency. This dog was humanely euthanized. A less severe disease was shown by the female dog, with a corresponding better expression of the protein at the immunohistochemistry. Surgical correction of a unilateral hind limb contracture resulted in a favorable three years follow-up.

Focal and systemic myositis were well represented in this case series and they were often related to very common infective disease, such as Leishmaniasis and Erlichiosis. Inflammatory myopathy associated with several infectious diseases occurs in dogs including those caused by *Toxoplasma gondii*, *Neospora caninum*, *Ehrlichia canis* and *Hepatozoon canis*. However, muscle disease due to *Leishmania* infection has been poorly documented. However it was demonstrated that, where *Leishmania* is an endemic disease, it should be considered as a cause of IM in dogs. *Leishmania* is not present within muscle fibers but in macrophages, and that the muscle damage might be related to immunological alterations associated with *Leishmania* infection. (Paciello et al. 2009)

In one case, Erlichiosis was related to a focal painful myositis and clinical remission result after tetracyclines therapy.

Other causes of focal disease were traumas, reported by the owner at the history.

In a case of semitendinosus m myopathy trauma was supposed as cause of the

muscle damage, but histopathology was suggestive of a neurogenic disease. Gracilis and/or semitendinosus myopathy has been reported sporadically in dogs, most commonly in male German Shepherds, with an age range from 8 months to 9 years. The etiopathogenesis of the disease is unclear even if it is generally related to a muscle injury from excessive activity, that can lead to muscle strain, with a sequence of inflammation, edema, localized hemorrhage and, eventually, fibrosis. (Braund 2006; Lewis D.D. 1997; Pettit G.D. 1978; Bennet A.R. 1986; Moore R.W. 1981; Capello V. 1993)

The examination of muscle biopsies, collected very early in the course of the disease could clarify the etiopathogenesis of this myopathy, that in human medicine is considered on a congenital basis or post-injective. (Gao G.X. 1988; Louis E.D. 1994; Van de Bergh 1997)

SIC, contractural syndromes and laxity shared with neuromuscular disorders many clinical features. The biopsied cases in our series gave not conclusive results. Even if in some cases aspecific or specific alterations were demonstrated, it was not always possible to define if they were primary or secondary. However electrophysiological and histopathological examination of a large series of cases could allow a better understanding of these syndrome, that could be the clinical expression of etiologically different diseases.

In conclusion, clinical, orthopedic and neurologic examinations allows to suppose a diagnosis of neuromuscular disorders. Whenever possible, in this cases only a diagnostic iter comprehensive of hematochemical analysis, electrophysiological examinations and biopsy is indicated to achieve a definitive diagnosis.

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